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EFFECTS OF LOCALIZED VENTRICULAR PERFUSION OF OUABAIN ON DORSAL HIPPOCAMPUS

Timothy Asbury Pedley

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EFFECTS OF LOCALIZED VENTRICULAR PERFUSION OF OUABAIN ON THE SPONTANEOUS ELECTRICAL ACTIVITY AND POTASSIUM INDUCED PAROXYSMAL ACTIVITY IN DORSAL HIPPOCAMPUS

by

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B. A. Pomona College 1965

A thesis submitted to the faculty in partial
fulfillment of the requirements for the degree

Doctor of Medicine

Yale University School of Medicine
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1969

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To acknowledge the vast amount of assistance, support and encouragement I have received from so many people over the past three years is for me at once both a presumption and a privilege: a presumption to suppose that from anyone less than a Shakespeare mere words could do full honor to those who have participated in the various stages of this project; and a privilege to be able to convey in some measure my gratitude to those who have made this undertaking worthwhile.

To attempt to thank Dr. Emil Zuckermann is an exercise in futility. His constant attention and patience went far beyond what was strictly demanded of him as a thesis advisor. As a mentor in experimental methodology in general and neurophysiology in particular, he was unexcelled. Whatever merit this work finds stems only from my association with him.

Dr. Gilbert Glaser has generously provided equipment and space. His thoughtful suggestions and helpful discussion have contributed much to the shaping of this dissertation. And to him I owe the interest I have in a career in neurology.

And finally, this work could not have been accomplished without the presence and willing help of the technical staff. This includes Colleen McCullough and Karen Ring who assisted in the laboratory, Frank Esposito who gave invaluable aid with recalcitrant electronic equipment, Helen Siwek who made the histological sections, Marie Pisano who helped with the flame photometry and Sherry Randall who did a heroic job of typing.

tap

Ach, was in tiefer Brust uns da entsprungen,
Was sich die Lippe schüchtern vorgelallt,
Missraten jetzt und jetzt vielleicht gelungen,
Verschlingt des wilden Augenblicks Gewalt.
Oft, wenn es erst durch Jahre durchgedrungen,
Erscheint es in vollendeter Gestalt.
Was glänzt, ist für den Augenblick geboren,
Das Echte bleibt der Nachwelt unverloren!

—— Goethe (FAUST)

INTRODUCTION

Shifts from the normal ionic gradients between intra- and extracellular compartments have been shown to occur during seizure activity induced by a variety of methods (19,58,66,93, 94,100), and it has been suggested that such changes altering the ratio of intra- and extracellular ion concentrations (principally sodium and potassium) may be a primary factor in producing the hyperexcitable state seen in neurons in an epileptic focus (91). The importance of ionic environment to paroxysmal activity in neuronal populations has been emphasized in recent studies by Glaser (37) and Zuckermann and Glaser (102).

The most persuasive and detailed studies on the role of potassium and sodium in nerve excitation are the elegant series of experiments performed by Hodgkin and Huxley (46-48) on the isolated giant axon in Loligo. The ionic theory which they formulated has, with minor limitations, been supported by work on other excitable tissues and preparations including the central nervous system (13,23,56,91).

As an action potential is propagated, the nerve axoplasm accumulates sodium and loses potassium. For a single impulse, or even trains of many impulses in an isolated nerve, this change is so small that the membrane potential returns to its resting level solely on the basis of the sodium and potassium ion fluxes set up by their concentration and electrical gradients.* During prolonged activity _in vitro_, and presumably always _in vivo_ where the interplay of excitatory and inhibitory inputs — both subliminal and threshold — affects the membrane potentials of neuronal elements constantly, the ionic balance is assisted in returning to its so-called resting level by an active pump. This mechanism, involving the expenditure of energy, is directed toward the extrusion of sodium ions from the axoplasm against both an electrical and concentration gradient, and the influx of potassium ions. During sustained activity the pump is able to compensate for the accelerated fluxes secondary to successive firings by working at a greater rate. With prolonged intense activity, however, the pumping mechanism is overwhelmed leading to marked accumulation of sodium and loss of potassium intracellularly, an event signalled by delayed recovery and relative unresponsiveness of affected neuronal aggregates (9, 45,54).

---

*It has been calculated and subsequently substantiated by experiments using radioactive tracer ions, that only one potassium ion in 10^7 would have to be replaced by a sodium ion to produce a voltage change of 120 mV associated with an action potential (45,57).

The ionic changes associated with the sodium pump have been related to the metabolic processes of cells by the discovery of a sodium-potassium-magnesium activated adenosine triphosphotase (Na-K ATPase) which fulfilled all the requirements of an energy system responsible for active transport (84,85). If the equating of this enzyme and the active pump mechanism is correct, then it becomes of crucial importance in maintaining membrane stability in neurons. Results from a current investigation by Abdel-Latif, Brody and Ramati (1) testify to the intimate relationship between the Na-K ATPase and cerebral electrical events. These experimenters recorded the electroencephalogram from fetal and newly-born rats and determined brain ATPase activity at different gestational and post-partum ages. The onset of recordable cortical discharges coincided with an abrupt rise in the specific activity of the enzyme. The EEG became rhythmic and regular through the twelfth day after birth corresponding the Na-K ATPase reaching adult levels of activity.

The linkage of active transport to a specific enzyme has raised the possibility that interference with such a system might be a factor in seizure genesis (71,91). Although there is no direct evidence for altered ATPase activity in human epileptic foci (71), several experimental studies have implied that such a situation might exist. Pappius and Elliott (71) observed impaired sodium extrusion in epileptic foci from human cortex. It has been postulated that the effectiveness of diphenylhydantoin as an anti-

convulsant may be due principally to stimulation of the Na-K ATPase (90,91).* Such a hypothesis is based on studies of Woodbury (99) who found that in addition to raising the electroshock threshold, diphenylhydantoin was regularly associated with a 60% increase in the $[Na_o]/[Na_i]$ ratio. He interpreted this to mean that active sodium extrusion was stimulated by the drug. Samson (77) has suggested that the Na-K ATPase has another function in addition to maintaining ionic gradients. He proposes that inhibitory neurons achieve hyperpolarization of post-synaptic elements through activation of ATPase system. Lewin and McCrimmon (61) have shown that the ATPase activity in experimental epileptic lesions produced by freezing is higher than in control cortex. Furthermore, recent studies have reported on the ability of ouabain — a drug which is a potent inhibitor of active transport — to induce convulsive activity when introduced into cerebral tissue (7,20,64) or applied to the cortical surface (4).

The present study was designed to investigate further the action of ouabain in inducing a paroxysmal state. To approximate as closely as possible the situation achieved _in vitro_ with isolated slabs of cortex, it was decided to use a technique in which cannulas could be implanted in the inferior horn of the lateral ventricle

---

*Recent work by Rawson and Pincus (73) indicates that this may not be the case, as in their studies diphenylhydantoin _inhibited_ Na-K activated ATPase from microsomal fractions of rat and guinea pig brain and from homogenates of human cortex in anticonvulsant concentrations.

(102), thus permitting constant perfusion of a localized area of brain, viz. dorsal hippocampus, with varying concentrations of ouabain. While recognizing that some diffusion would occur away from the perfused region, it was hoped that successful placement of the electrodes close to the cannulas would allow careful monitoring of focal changes in electrical activity.

Chronic cats were used for the experiments to avoid effects of anesthetics and the acute trauma of the surgical implantation. Furthermore, each animal could serve as its own control and as the subject for repeated experiments, and any long-term electrographic changes could be followed.

MATERIALS AND METHODS

Experiments were performed on a total of forty-five cats. The technique described follows that used by Zuckermann and Glaser (102,103).

Surgery was performed under pentobarbital anesthesia, 38mg/kg administered intraperitoneally. Additional barbiturate was given as needed during the course of the operation. To decrease cerebral edema during and following surgery, intramuscular dexamethasone, 0.6mg/kg, was given periodically. Occasionally intraperitoneal urea was injected during an operation of unusual length or if the brain sustained trauma greater than was customarily encountered. The animals were prophylactically maintained post-operatively on benzathine penicillin G.

In all hippocampal preparations (34 cats), implantation of electrodes and cannulae for ventricular perfusion was carried out in a Horsley-Clarke stereotaxic apparatus using the coordinates of the Snider-Niemer atlas (87). Two or three 22-gauge stainless steel cannula, 28 millimeters in length were positioned at

A 2.5 - 3.5 and L 3 - 8, usually 2 - 3mm apart. Satisfactory placement in the inferior horn was assured by connecting the cannula to a manometer filled with artificial cerebrospinal fluid (see below) and adjusting the final height of the cannula until the fluid fell readily in the manometer and an increase in flow was observed from a silastic tubing temporarily inserted into the cisterna magna.

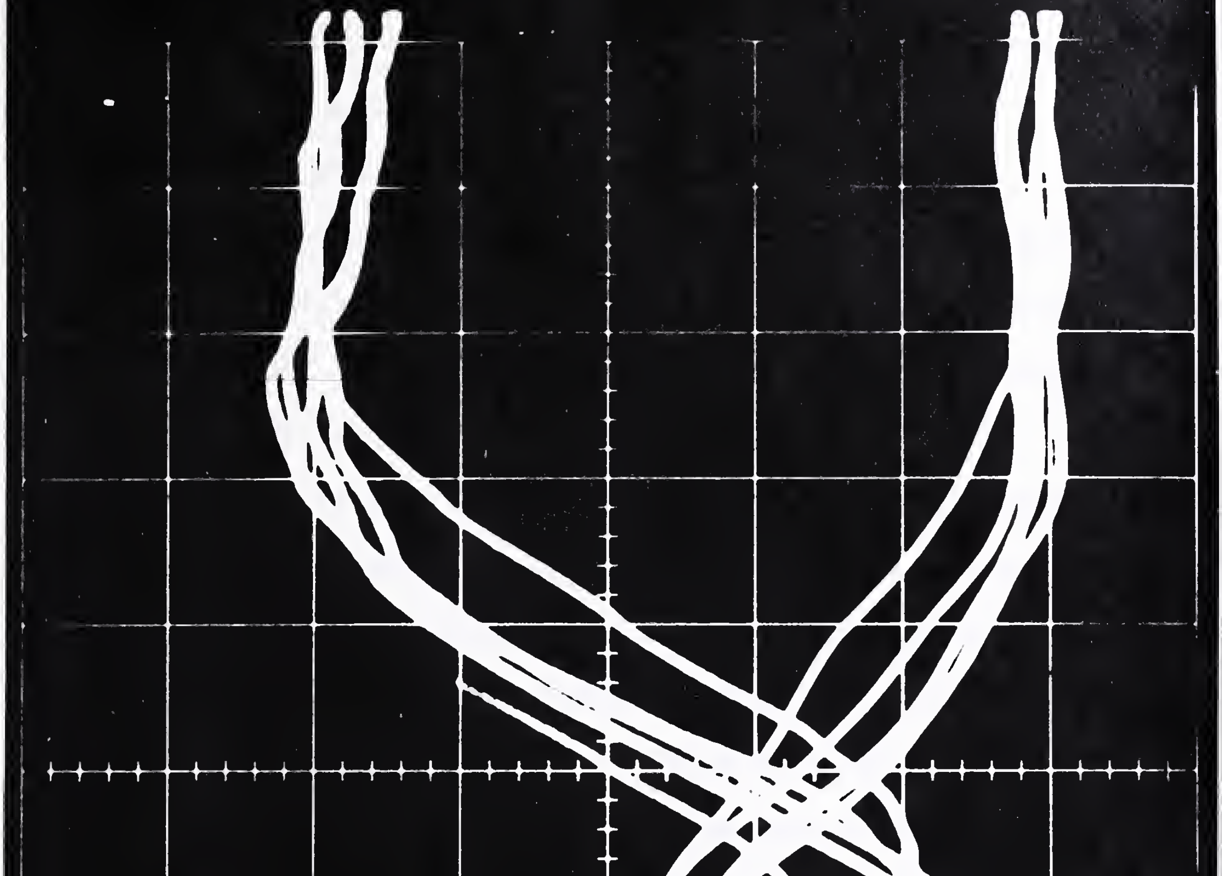
The electrodes used were coaxial pairs of Teflon-coated stainless-steel wires, 100 u in diameter and with a tip separation of 0.8 - 1.0mm. Four of these electrode pairs were placed in the CA1 and CA2 fields of right and left dorsal hippocampus. Priority in placement was given to achieving close proximity to the cannulas. The final vertical position for each electrode pair was critically made by stimulating contralateral hippocampus through a pair of electrodes simultaneously placed, or—subsequent to the initial placement—ipsilateral hippocampus through a pair of previously implanted electrodes and recording the commissural or local response. The electrodes were then lowered in increments until a characteristic pattern of evoked response was obtained (Fig. 1), signifying successful recording from apical and basilar dendrites of hippocampal pyramidal cells (3,30,42,103). Thus, electrical activity could be recorded from four different points in each hippocampus and from two heights at each point.

In eleven cats, a cortical preparation was made instead by placing a lucite chamber with an opening of 6mm on the cortex

Figure 1

Photograph of oscillographic tracing of commissural evoked response. This is typical of the pattern used to determine proper vertical placement of electrodes.

Upper tracing: apical dendritic layer
Lower tracing: basal dendritic layer
Calibration: each division 250uV and 10msec.
Negativity is upwards.



one centimeter behind the bregma and fixing it to the calvarium. Electrodes were then implanted one millimeter below the surface of gyrus lateralis and gyrus suprasylvius, and pairs of cannula for perfusion within the chamber were placed with their tips several millimeters above the cortical surface.

All wires were led through Amphenol connectors to an Amphenol plug, and the entire apparatus was securely fastened to the skull with dental cement. Radiographs of a living cat with the electrode assembly in place are shown in Fig. 2.

Animals were usually allowed to recover for two weeks before beginning an experimental series, but in a few instances, experiments were begun 4 - 7 days after the surgery. For each experiment the cat was put in a specially constructed "cat box" which restrained the cat in a normal lying position, allowing movement of the head only. Connections to the recording and stimulating equipment were made through the single Amphenol plug fixed to the cat's skull. Perfusion was established between two adjacent cannula, the inflow coming from a Harvard Infusion Pump through silastic tubing connected to the pump and to one cannula. The outflow was collected in a graduated test tube under negative pressure. A manometer in parallel with the inflow system indicated the pressure at which the perfusion fluid entered the ventricular system. This pressure was usually between 20 and 30cm. of water. Interposed between the pump and the inflow cannula was a water bath which raised the temperature of the perfusing fluid to 37°C. Although the perfusion rate could

Figure 2

Roentgenograms of a living cat demonstrating the electrode assembly in place. From left to right: anteroposterior, straight - on vertex, and lateral views.

be regulated somewhat by changing the perfusion pressure within the limits noted, or by varying the intensity of the negative pressure applied to the outflow cannula, it fluctuated from one experiment to another, and from cat to cat. Most often, however, it remained constant during a single experiment. Rates of perfusion from 0.025ml/min. to 0.47ml/min. were observed.

As a measure of hippocampal excitability, a Grass Model S-8 or a Grass Model S-4 stimulator was used with a Grass Stimulus Isolation Unit to deliver a train of 10 biphasic pulses over 12.5 seconds every 2 - 5 minutes during all perfusions. Each pulse had a duration of 0.08msec. and the intensity used was determined in one of two ways. The first method involved increasing the voltage in increments until a small motor response was observed (e.g. an ear twitch, eye blink, etc.) with each stimulus. An alternative method was to determine the voltage-frequency combination which when applied as a rapidly repetetive train of biphasic pulses over 12.5 seconds would produce a minimal electrical seizure. This voltage value was then arbitrarily doubled and used as the intensity for the "single-shock" trains delivered during perfusion. Electrical activity was recorded on either a Grass Model III-A Electroencephalograph or a Grass Model 7 Polygraph.

In the first series of experiments, each session usually began with a 45 minute perfusion of "normal" cerebrospinal fluid (Table 1) during which single shocks as detailed above were delivered through one pair of electrodes. Artificial cerebrospinal fluid with

TABLE 1

Composition of Artificial Cerebrospinal Fluid

NaCl	126.5mM/L
KCl	3.0
$CaCl_2$	2.3
$MgCl_2$	0.8
NaH_2PO_4	0.5
$NaHCO_3$	13.2

The pH of all solutions was adjusted before use to 7.3 - 7.4. (After Zuckermann and Glaser 102)

TABLE 2

Concentration of potassium in high-potassium cerebrospinal fluid

K X 2.0	6.0
K X 3.0	9.0
K X 4.5	13.5
K X 6.0	18.0
K X 12.0	36.0
K X 15.0	45.0
K X 18.5	55.0
K X 19.5	58.5
K X 22.0	66.0

In all solutions containing greater than 18.0meq. of potassium, K_2SO_4 rather than KCl was used in making up the solutions to reduce the swelling associated with high concentrations of chloride (11). Any increase in potassium was accompanied by a corresponding decrease in sodium to maintain isomolarity between all solutions.

ouabain* in concentrations of 10^{-7}M to 10^{-4}M was then perfused. Repeated perfusions of gradually increasing ouabain strength were made until an electrographic change was observed. Each perfusion lasted 45 minutes and at least 20 minutes was allowed to elapse between perfusions. If a change in the electrical activity was seen, the perfusion was stopped and normal cerebrospinal fluid perfused immediately in an attempt to remove excess ouabain.

In an effort to define more clearly a quantitative relationship between ouabain and changes in the spontaneous electrical activity, a second group of hippocampal-implanted cats was used. As there was no readily available assay to determine ouabain concentrations in the effluent as compared to the inflow, and because of the unavoidable variation in perfusion rates, it was decided to titrate known amounts of ouabain against changes in the electrical activity. This was done by injecting amounts from 2 to 20ugm through a cannula into the inferior horn. In an attempt to avoid any cumulative effects of the drug which might obscure the results, a given cat was infused with only a single dose during any one experimental session. Furthermore, in these experiments, a minimum of ten days was waited between sessions.

---

* The ouabain used was crystalline ouabain, USP (Mol. Wt. 728.8) and was obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Final concentrations were made by diluting a stock solution of 10^{-3}M in artificial cerebrospinal fluid.

A third series of experiments was carried out to ascertain the relationship of ouabain to the potassium-induced paroxysmal activity. In each of these experiments, artificial cerebrospinal fluid with increasing concentrations of potassium (Table 2) was perfused first until seizure activity was induced with single shocks, thus determining the "potassium threshold." Ouabain in concentrations below those which had produced electrical changes was then added to the perfusion fluid containing either that concentration of potassium which had induced paroxysmal activity or an amount about 30% below it. The effect of the two drugs together on hippocampus as manifested by electrographic changes was then studied. A final perfusion was made at the end of each experiment with artificial cerebrospinal fluid containing ouabain alone in the concentration it had been used in conjunction with potassium to assure that indeed, it was an amount that by itself induced no apparent change in electrical activity. In a few additional experiments, high-potassium cerebrospinal fluid was perfused at different intervals after perfusion with subconvulsive concentrations of ouabain rather than concomitantly.

The cats with cortical implantations were used for a similar set of studies in order to compare cortical and hippocampal response to ouabain and potassium. Also, it was hoped that these animals would provide a preparation where precise control of perfusion rate could be obtained and further quantification of results made. Each cat served initially to determine the

concentration of ouabain necessary to induced epileptic activity. After a one-week interval, a second round of experiments was begun. In a typical session, determination of the potassium threshold was performed, followed by a second perfusion to confirm it. During the first perfusion, single shocks were delivered. No stimulation was done during the second perfusion of potassium. Then this concentration of potassium was perfused together with a subconvulsive concentration of ouabain. Finally, perfusion with ouabain alone in the concentration it had been used with potassium was made.

At least four days after electrical and behavioral activity had returned to normal was allowed to elapse between experiments on the same animal. Zuckermann and Glaser found this delay between sessions appeared to insure hippocampal excitability returning to its previous level (102).

The effluents collected during perfusion were analyzed if sufficient in amount by means of a Process and Instruments Corporation Flame Photometer. The concentrations of potassium and sodium were determined and compared with the concentrations of these ions in the perfusion fluid as it entered the subarachnoidal space.

At the end of an experimental series on a hippocampal preparation, the cat was anesthetized with pentobarbital. Electrolytic lesions marking the electrode positions were made by passing a DC current through each electrode. The animal was

then killed by intracardiac perfusion with saline and 10% formalin. The brain was sectioned and alternate 15 - 20u slices stained with thionin, Weil's method or Gomori's iron reaction to verify electrode placement and to examine for pathological changes. The brains from cats with cortical implantations were only examined grossly.

A summary of the experimental protocols is given in Fig. 3.

Fig. 3

SUMMARY OF EXPERIMENTAL PROTOCOLS

Surgical implantation of 45 cats

34 Hippocampal Preparations

A. Perfusion with ouabain alone
   Successive perfusions of increasing ouabain strength beginning with 10^{-7}M

B. Infusion of known amounts of ouabain
   2-20ugms injected into the inferior horn of different cats

C. Perfusion with ouabain and potassium in combination
   1. Perfusion with potassium alone
   2. Perfusion with either subthreshold potassium or threshold potassium together with a subconvulsive concentration of ouabain
   3. Perfusion with the concentration ouabain used alone

↓

Analysis of Perfusates

↓

Histologic examination of brains

11 Cortical Preparations

A. Perfusion with ouabain alone

B. Perfusion with ouabain and potassium together
   1. Perfusion with ouabain alone
   2. Perfusion with threshold potassium in combination with subconvulsive concentrations of ouabain
   3. Perfusion with ouabain alone in the concentration used

↓

Gross post-mortem examination of cortex

RESULTS

I. Hippocampal Preparations

A. General Behavior

The cats adapted well to the restraints imposed by the boxes. After a brief period of restlessness they settled down, often going to sleep during much of the experiment. Perfusion with normal cerebrospinal fluid and subconvulsive concentrations of ouabain and/or potassium did not affect the animal's gross behavior in that they remained alert (or easily arousable if asleep) and reactive to environmental stimuli. Alterations in behavior seen with potassium-induced paroxysmal activity were similar to those described elsewhere (102). With ouabain in concentrations producing localized changes in electrical activity, a decreased alertness and unresponsiveness to provocative stimuli (e.g. loud, sudden sounds, petting, menacing gestures) preceded any signs of motor activity. Often hyperpnea and tachypnea heralded an imminent convulsion. As seizure activity spread to contralateral hippocampus and became generalized, the animals

began licking their lips, sniffing and exhibiting focal muscular twitches (usually ear, facial or whisker). If the paroxysmal activity still increased in intensity, the onset of tonic-clonic convulsions with aggressive biting of the cat box and often fecal and urinary incontinence was seen. If the animal survived, its behavior remained altered up to 48 hours after the end of an experiment, with the cat sitting crouched and unresponsive in its cage not eating or drinking. Occasionally, mildly aggressive attitudes were noted during this period. After this time, the behavior returned to normal and the cat acted as it had before the experiment.

B. Changes in the spontaneous hippocampal electrical activity.

1. Perfusion with normal cerebrospinal fluid

The low amplitude fast activity of 18 - 20/sec alternating with high amplitude slow waves 4 - 5/sec described in detail elsewhere (40,41) was taken as the normal background activity and used as the baseline for comparison with any changes induced by the perfusions.

In 58 perfusions with normal cerebrospinal fluid for 45 minutes, no changes in the recorded electrical activity were observed. Stimulation with single shocks likewise produced no changes in the electrographic patterns.

2. Perfusion with cerebrospinal fluid containing ouabain

In 16 experiments on 10 cats, ouabain consistently induced seizure activity in concentrations greater than 5 X 10^{-6}M. In no animals were electrographic changes recorded with perfusions for the standard 45 minute period containing 10^{-6}M ouabain or less.

In 10 of these experiments on seven cats, a characteristic pattern was observed (Fig. 4 & 5). This was typified by a slow build-up of increasing electrical activity until a generalized seizure developed. Usually spikes occurring regularly from one area of the perfused side spread until the entire perfused side was discharging synchronously at 1 - 3/sec. This low frequency spiking then increased in both frequency and amplitude, spreading contralaterally and becoming generalized. Termination of the paroxysmal activity was marked by regular bursts of spike complexes, each complex composed of 4 - 6 high amplitude (300 - 500uV) spikes. Similar bursts were often seen preceding the generalized seizure as well (Fig. 5).

In 4 of the experiments on 3 cats, a slight variation was noted. Rather than being preceded by a slow build-up, a continuous discharge of synchronized spikes 8 - 13/sec. with an amplitude of 200 - 500uV was seen to begin immediately. In these cases, the seizure ended abruptly after 30 - 90 seconds with the return of the electrical activity to normal with some occasional depression.

Figure 4

Cat X. Perfusion with 5 X 10^{-6}M ouabain.

A.) Onset of paroxysmal activity 15 minutes after beginning the perfusion. Spikes are limited to the perfused dorsal hippocampus.

B.) Increase of spike frequency and amplitude and the appearance of epileptic activity on the contralateral side 20 seconds later.

C.) Termination of generalized seizure 2 minutes after the appearance of the first spike.

In this, and in all subsequent electrographic records, the position of the electrodes (solid dots with numbers) and the cannulae (open circles without numbers) is shown in the diagram in relation to certain hippocampal and neighboring anatomic structures. The view shown is a dorsal one, with the inferior horns lying immediately above the hippocampi. The small numbers to the right and bottom of the diagram give the approximate stereotaxic coordinates of the electrodes and cannulae. Redrawn from Andersen in Acta Physiol. Scand. (1959) 47:65

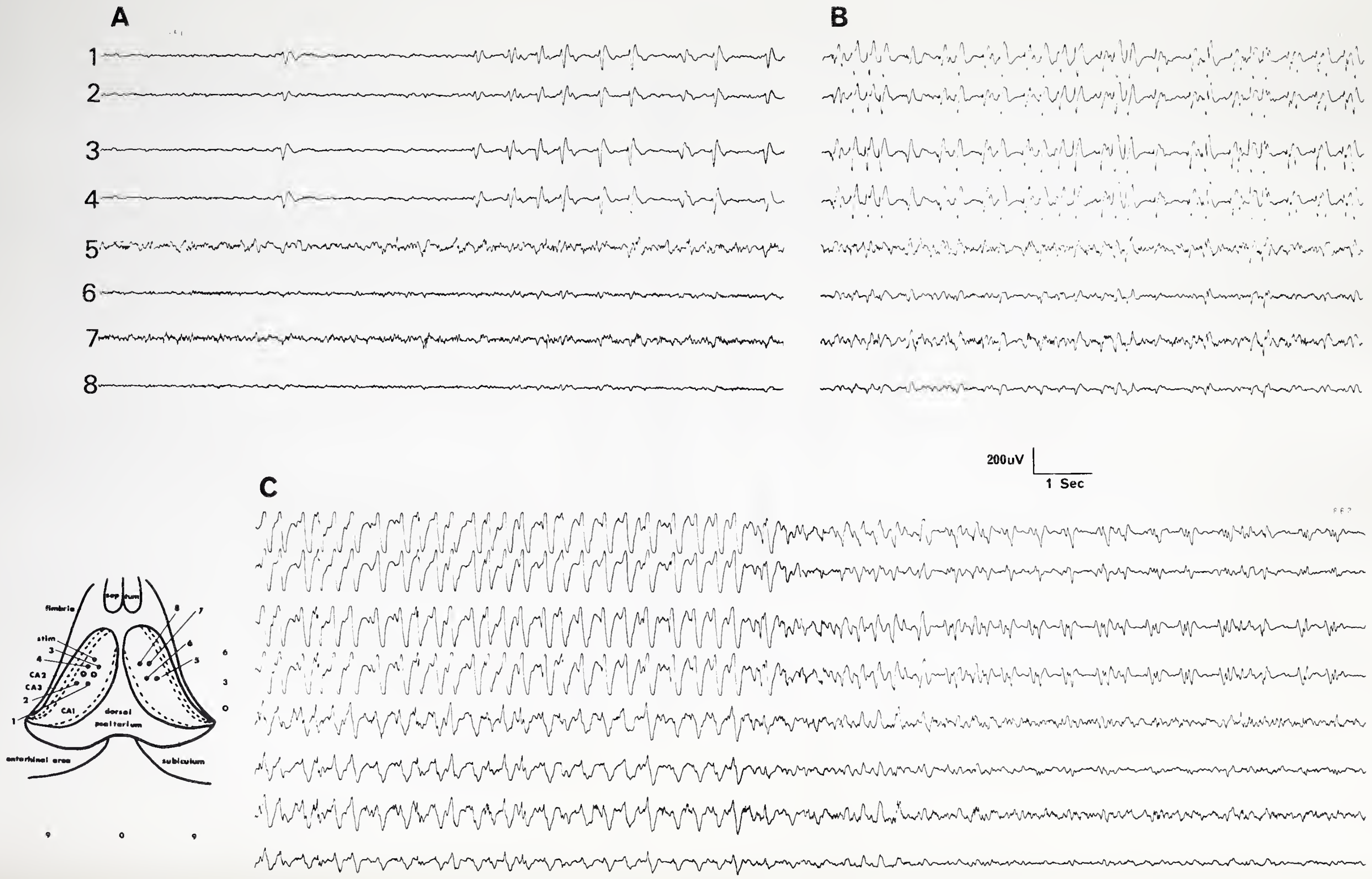

A
1
2
3
4
5
6
7
8
B
200uV
1 Sec
C
fimbria
septum
stim
3
4
CA2
CA3
2
1
CA1
dorsal
psalterium
8
7
6
5
entorhinal area
subiculum
6
3
0
9
0
9

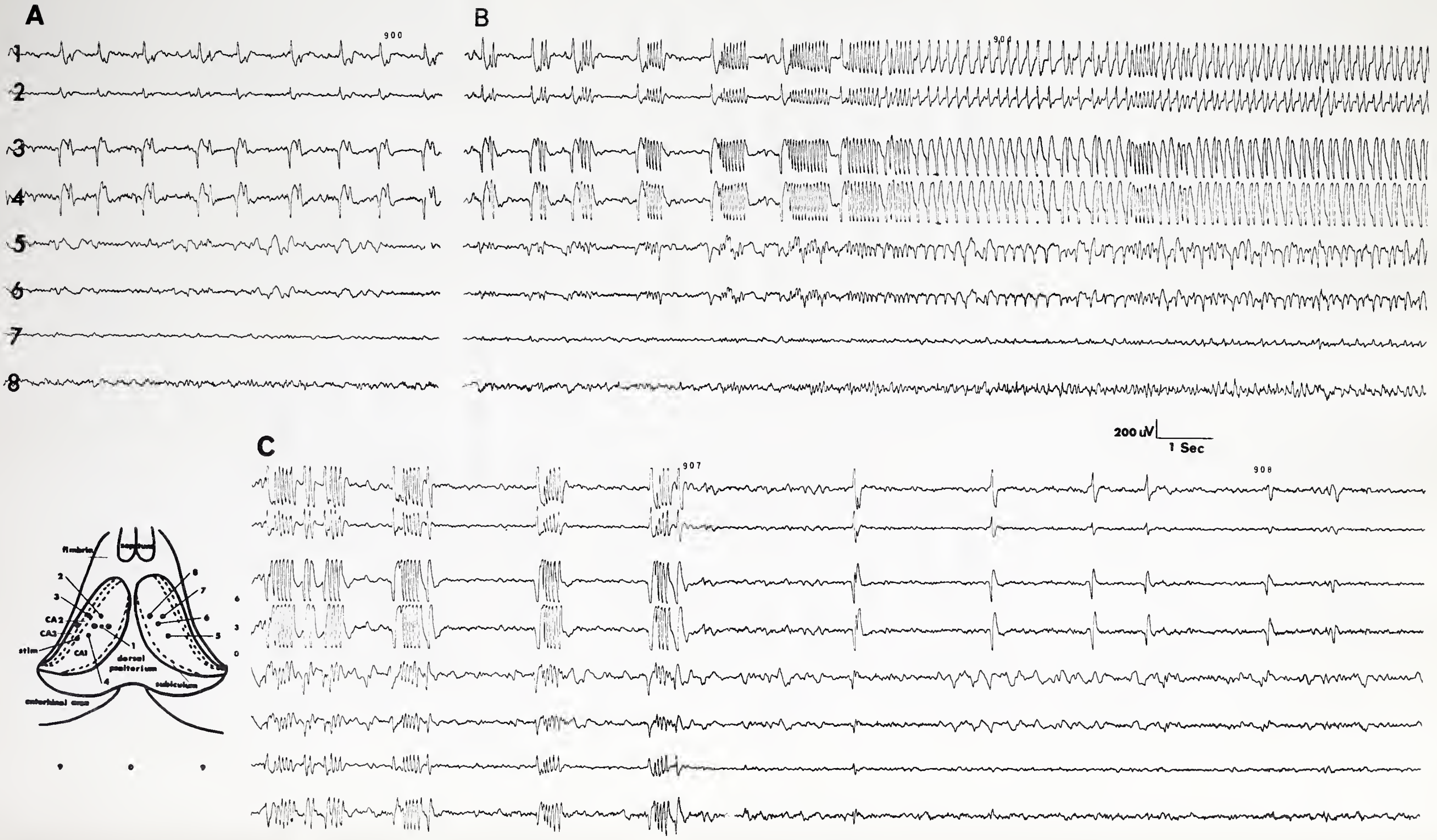
Figure 5

Cat VIII. Perfusion with 10^{-5}M ouabain.

A.) Onset of spike activity on the perfused side 18 minutes after beginning the perfusion.

B.) Appearance of bursts of spikes leading to continuous paroxysmal activity. Some contralateral spread is also seen. 40 seconds after appearance of first spike.

C.) Termination of seizure 80 seconds after the appearance of the first spike.


A
B
C
1
2
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4
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8
900
904
907
908
200 uV
1 Sec
fimbria
2
3
CA2
CA3
stim
CA1
4
1
dorsal psalterium
subiculum
entorhinal area
8
7
6
5
6
3
0

In all but one of the experiments, regardless of the electrographic pattern, a second seizure identical to the first developed within 10 minutes of the initial one. And in 5 experiments, seizures continued to occur, the interictal periods decreasing until continuous paroxysmal activity was present, terminating only with the animal's death 1 - 2 hours later.

With one exception, stimulation of the perfused dorsal hippocampus with single shocks (See Methods) did not induce paroxysmal activity, all seizures appearing to arise independently of stimulation. The one exception was most likely a coincidence with the onset of the seizure happening to occur between two pulses. Whether the single shocks lowered the thresholds of the perfused neuronal elements cannot be inferred from these experiments as the latency of onset of paroxysmal activity varied widely at a given ouabain concentration from experiment to experiment.

In 75% of the experiments, the seizure focus was clearly on the perfused side with projection to the contralateral hippocampus and secondary spread to other cortical tissues. In 25% (the 4 experiments described above as variants of the usual ouabain-induced electrographic pattern) the focus could not be clearly determined as the seizure activity appeared in all leads at once.

3. Infusion of known amounts of ouabain into the inferior horn

Twenty-six experiments were performed on 15 cats. The results are summarized in Table 3, and a graph depicting the inverse relationship between latency of onset of paroxysmal activity and the amount of ouabain used is shown in Fig. 6. As eight of the cats died after the initial experiment, the other seven cats served for a total of 18 experiments.

In no animal were electrographic changes observed with less than 3ugms of ouabain. In all experiments in which 4ugms or greater was given, the pattern of paroxysmal activity was virtually the same as that described above in Section 2. For comparison, an entire sequence is shown in Fig. 7. Stimulation of dorsal hippocampus with single shocks after the infusion of ouabain did not appear to affect the onset of seizure activity.

C. Effect of ouabain on potassium-induced paroxysmal activity

The interaction of ouabain and potassium was studied in 12 experiments on 8 cats. Three cats demonstrated seizure activity with high-potassium cerebrospinal fluid at 4.5 times normal and 5 cats required cerebrospinal fluid containing 6 times normal potassium to provoke epileptic activity. In all cases, single shock trains were effective in inducing well-organized epileptic discharges from the perfused hippocampus (Fig. 8).

TABLE 3

Amount ouabain ugms.	No. expts.	No. cats	No. cats surviving	Time of death after infusion (hours)	Appearance of first spikes (minutes)	No. expts. showing epileptic activity
2.0	2	2	2	---	---	0
2.5	2	2	2	---	---	0
3.0	3	3	3	---	35 - 40	2
3.5	3	3	3	---	30 - 40	2
4.0	5	4	4	---	15 - 25	4
5.0	5	5	3	12	15 - 18	5
7.0	2	2	0	12	10 - 15	2
10.0	2	2	0	2 - 5	5 - 6	2
15.0	1	1	0	1	5	1
20.0	1	1	0	1.5	2	1

Figure 6

Depiction of the inverse relationship between amount of ouabain and the time of onset of epileptic activity. Since not sufficient experiments were performed to enable a true mean to be calculated, the low and high limits for onset of paroxysmal activity have been indicated at each amount ouabain used. A line has then been arbitrarily drawn between the midpoints of these limits for illustrative purposes.

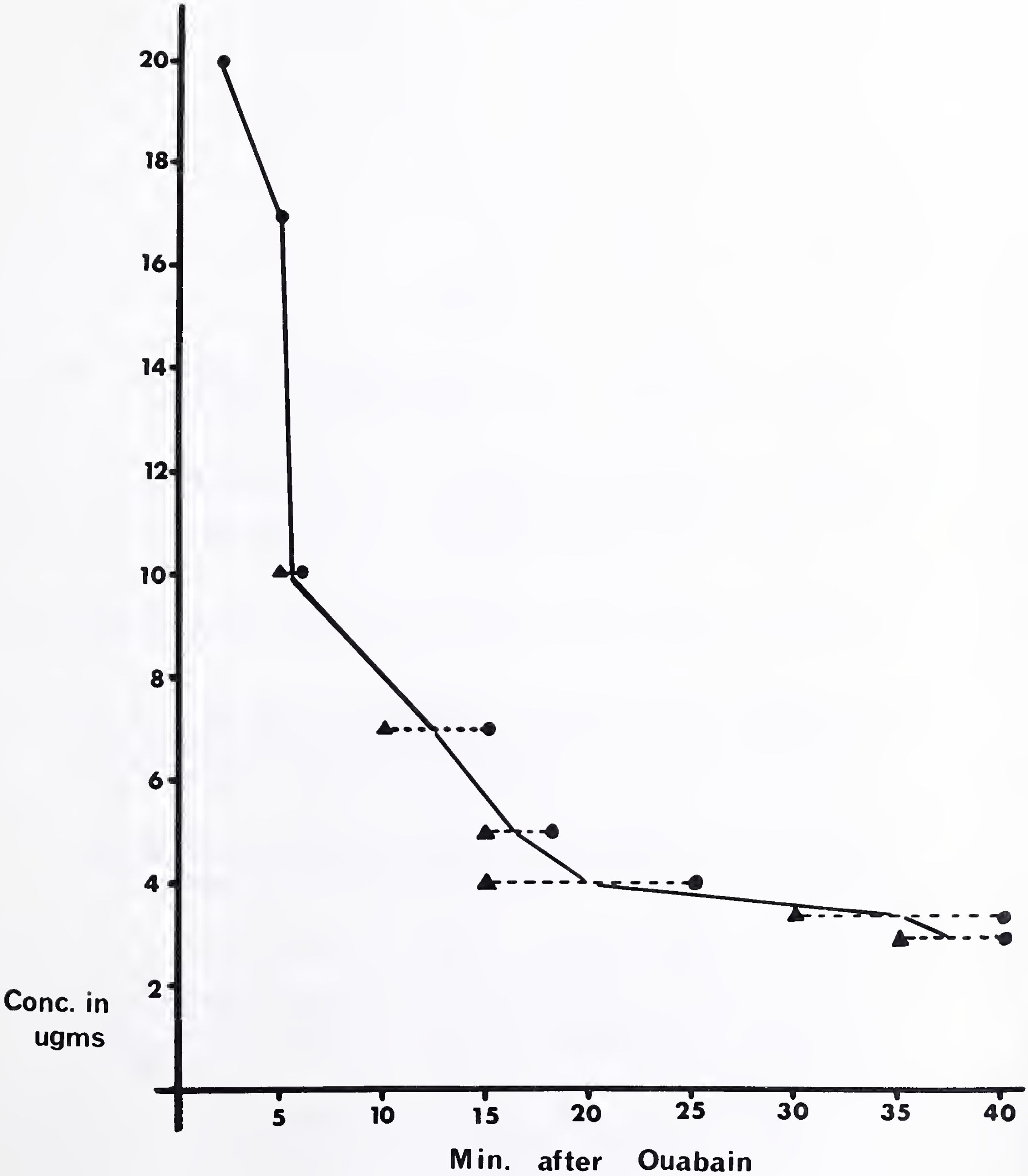

20
18
16
14
12
10
8
6
4
2
Conc. in ugms
5
10
15
20
25
30
35
40
Min. after Ouabain

Figure 7

Cat 48. Infusion of 5ugms. ouabain into the inferior horn of the lateral ventricle.

A.) Normal background electrical activity before the infusion.

B.) Onset of spike activity 18 minutes after the ouabain was injected.

C.) Lack of effect of single shocks on the emerging epileptic activity.

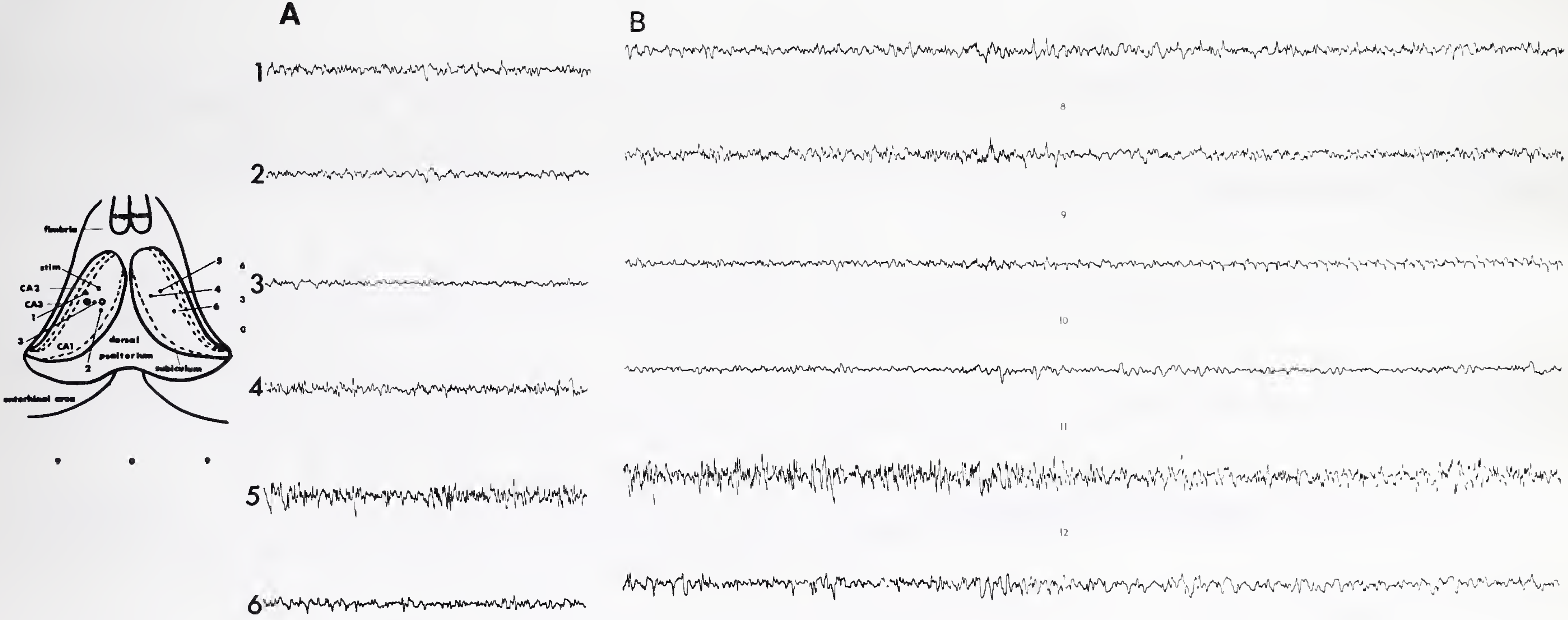
D.) and E.) Spread of paroxysmal activity to all leads of the ipsilateral side, increasing frequency and amplitude 24 minutes after infusion of ouabain.

F.) Contralateral spread has occurred and the cat has begun to show some clinical signs of the seizure: dilation of pupils, blinking and ear twitching.

G.) Generalized convulsion begins 38 minutes after ouabain.

H.) Termination of epileptic activity 43 minutes after infusion showing intermittent bursts with depression of background activity.

I.) Interictal depression with onset of a second seizure.


A
B
fimbria
stim
CA2
CA3
1
3
CA1
2
dorsal
psalterium
subiculum
entorhinal area
5
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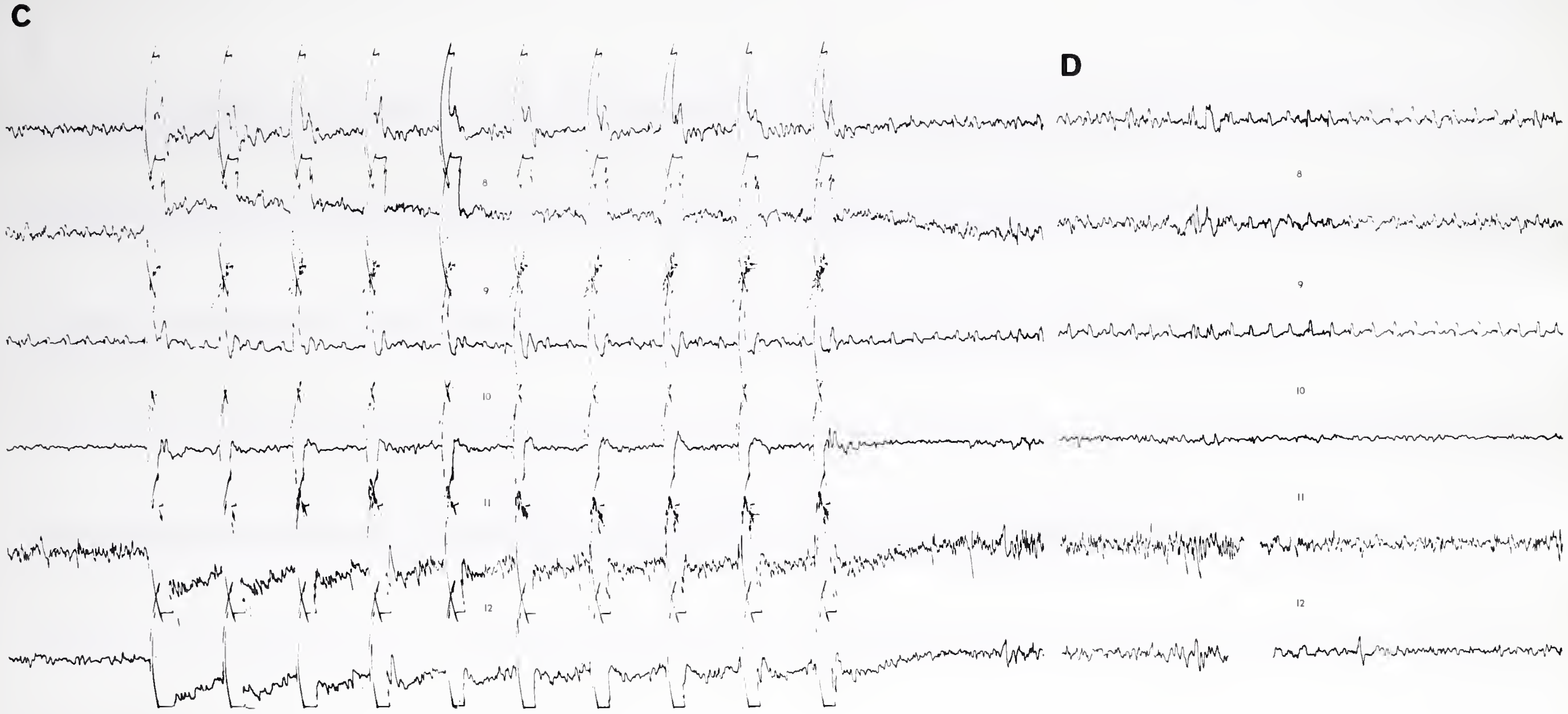
12


C
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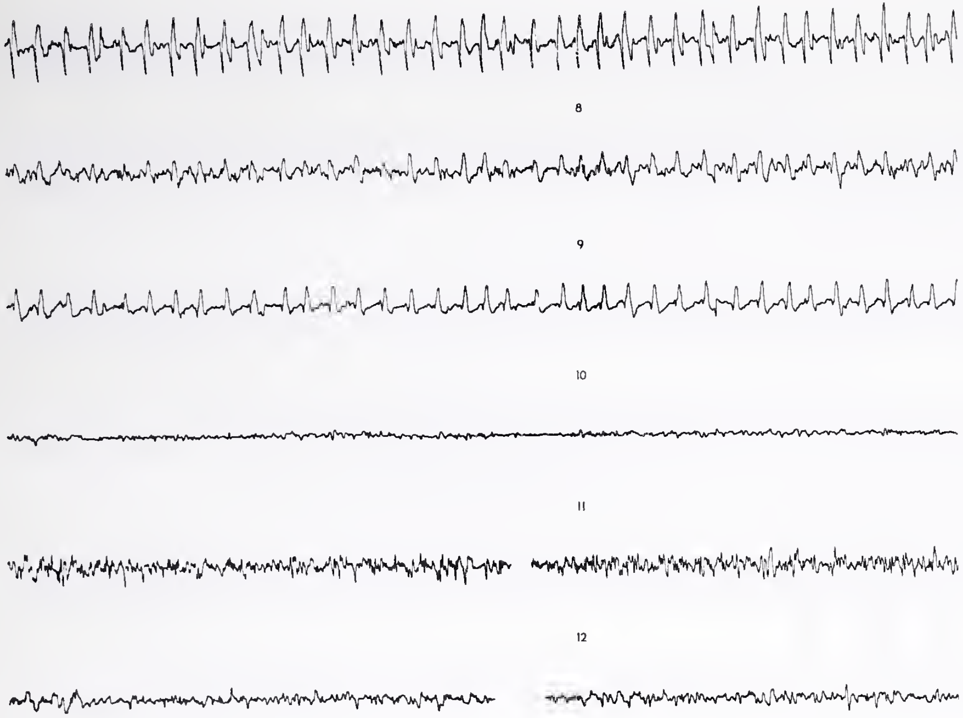
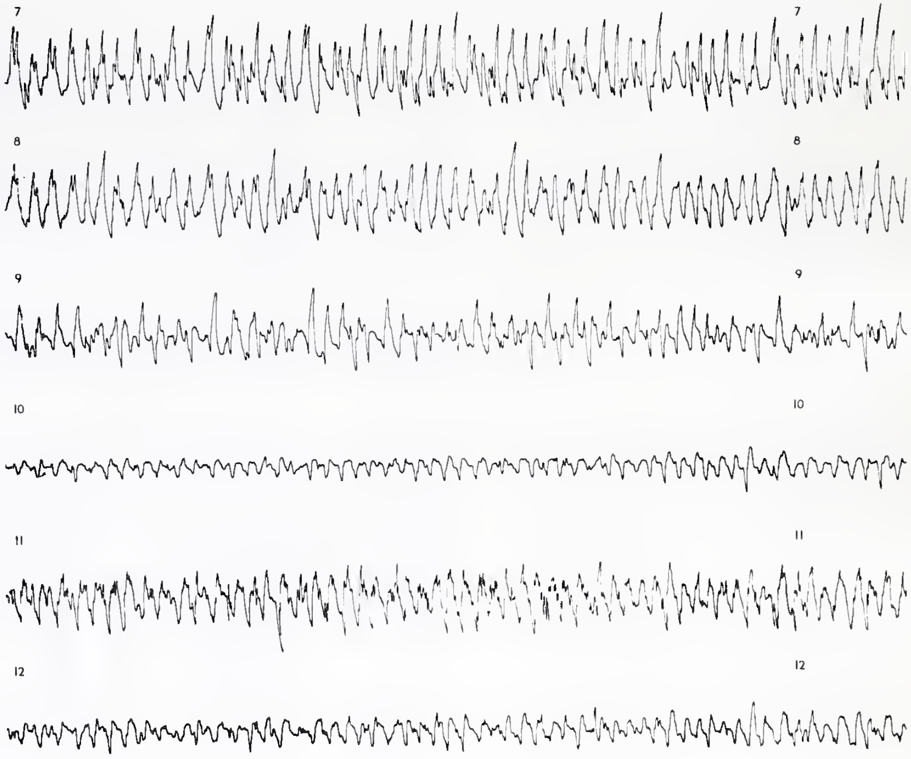
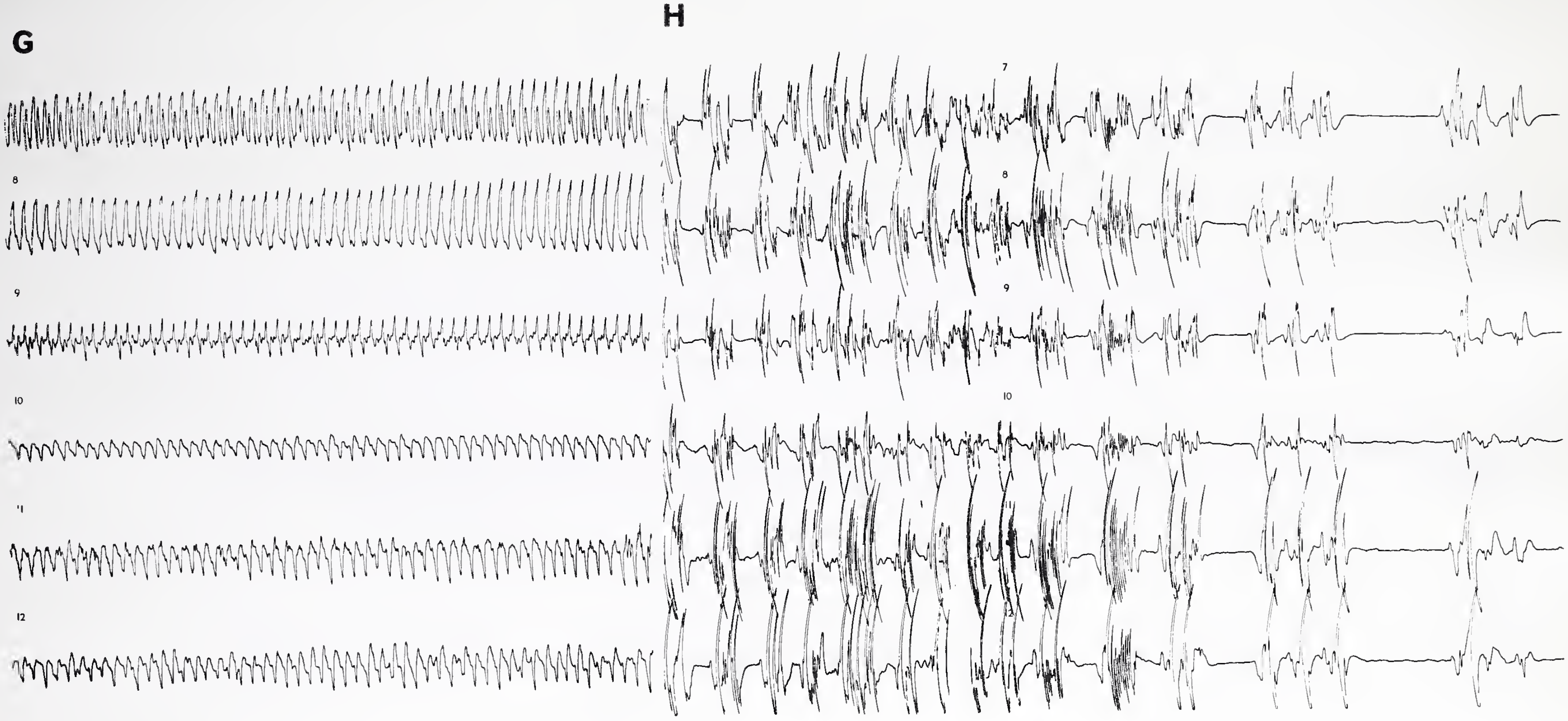


F

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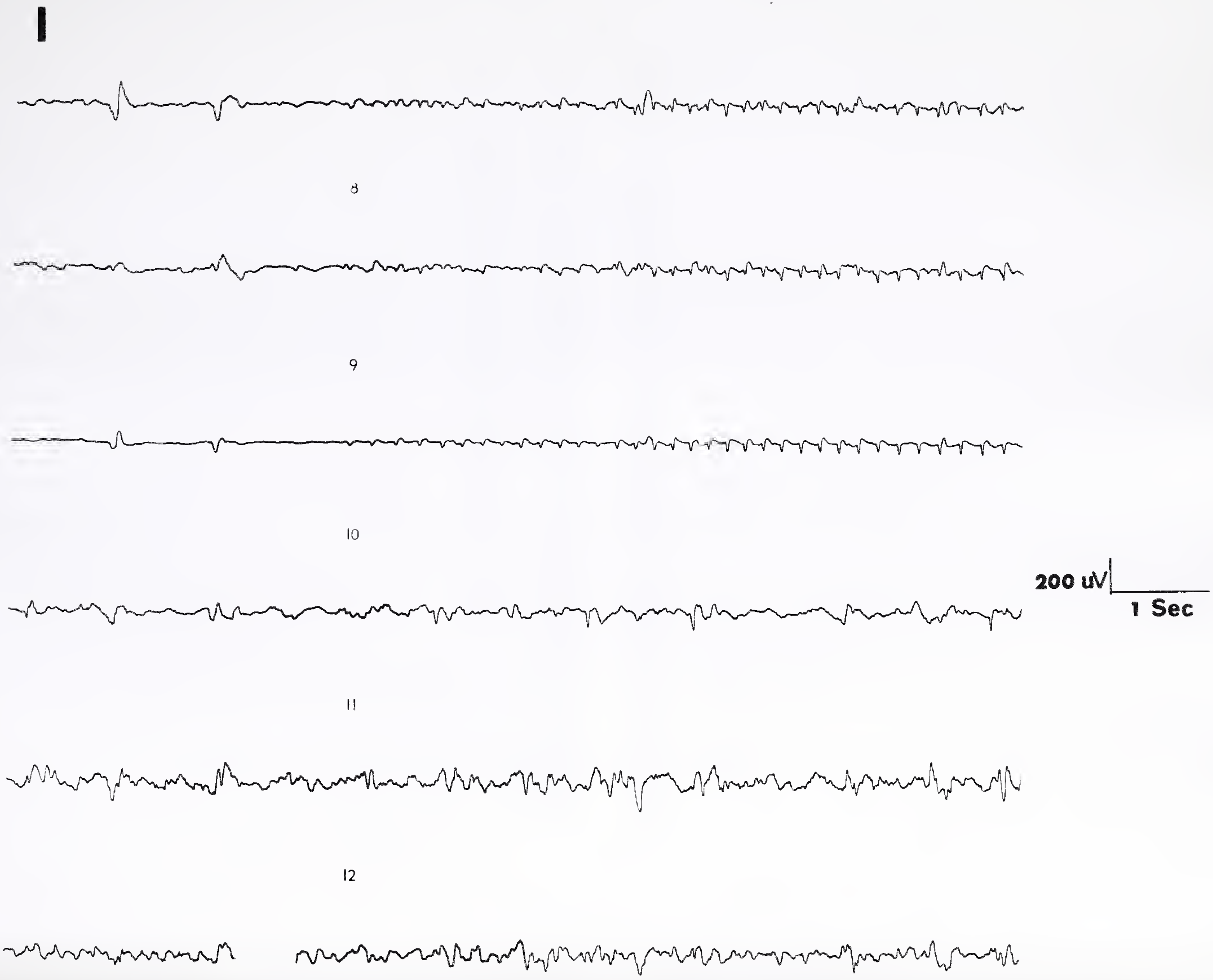
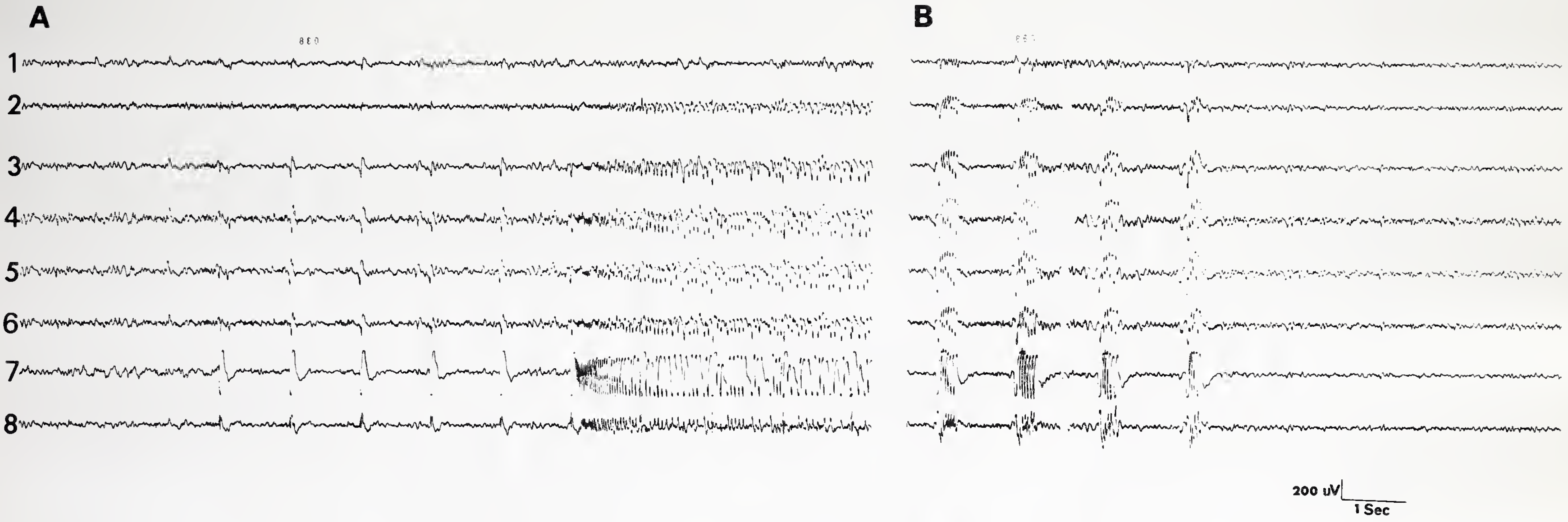

8
9
10
11
12
200 uV
1 Sec

Figure 8

Cat 10. Perfusion with high-potassium (18meq/L) cerebrospinal fluid.

A.) Onset of paroxysmal activity 12 minutes after beginning of perfusion. The seizure is induced by single shocks.

B.) Termination of seizure 50 seconds later.


A
B
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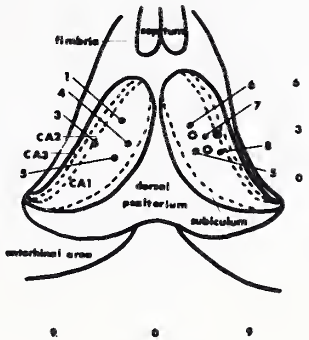
2
3
4
5
6
7
8
200 uV
1 Sec


septum
fimbria
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4
3
CA2
CA3
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CA1
dorsal
psalterium
subiculum
entorhinal area
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8
5

In five experiments on four cats, ouabain in sub-convulsive concentrations (10^{-6} to 2 X 10^{-6}M) was added to subthreshold concentrations of high-potassium cerebrospinal fluid. In all instances seizure activity was induced with single shocks (Fig. 9). In one experiment the seizure became more and more intense culminating in the animal's death. In no experiment did potassium alone in the concentration used lead to epileptic discharges. And in only one experiment did the ouabain, when perfused alone at the end of the session, lead to paroxysmal activity. This occurred without inducement by a single shock train.

The seizure pattern recorded with ouabain and potassium together corresponded closely to that observed with potassium alone. Continuous spikes at a frequency of 18 - 30/sec began abruptly, either appearing to arise from the decaying stimulus artefact or following closely thereafter. The rapid synchronous firing gradually decreased in frequency with the seizure terminating in regular bursts of discharges composed of 4 - 6 high amplitude spikes. In all experiments, at least one additional seizure occurred at a 5 - 10 minute interval after the termination of the first. Single shocks could occasionally elicit brusts of spikes coincident with the stimulus pulse up to 15 - 20 minutes after termination of the last seizure.

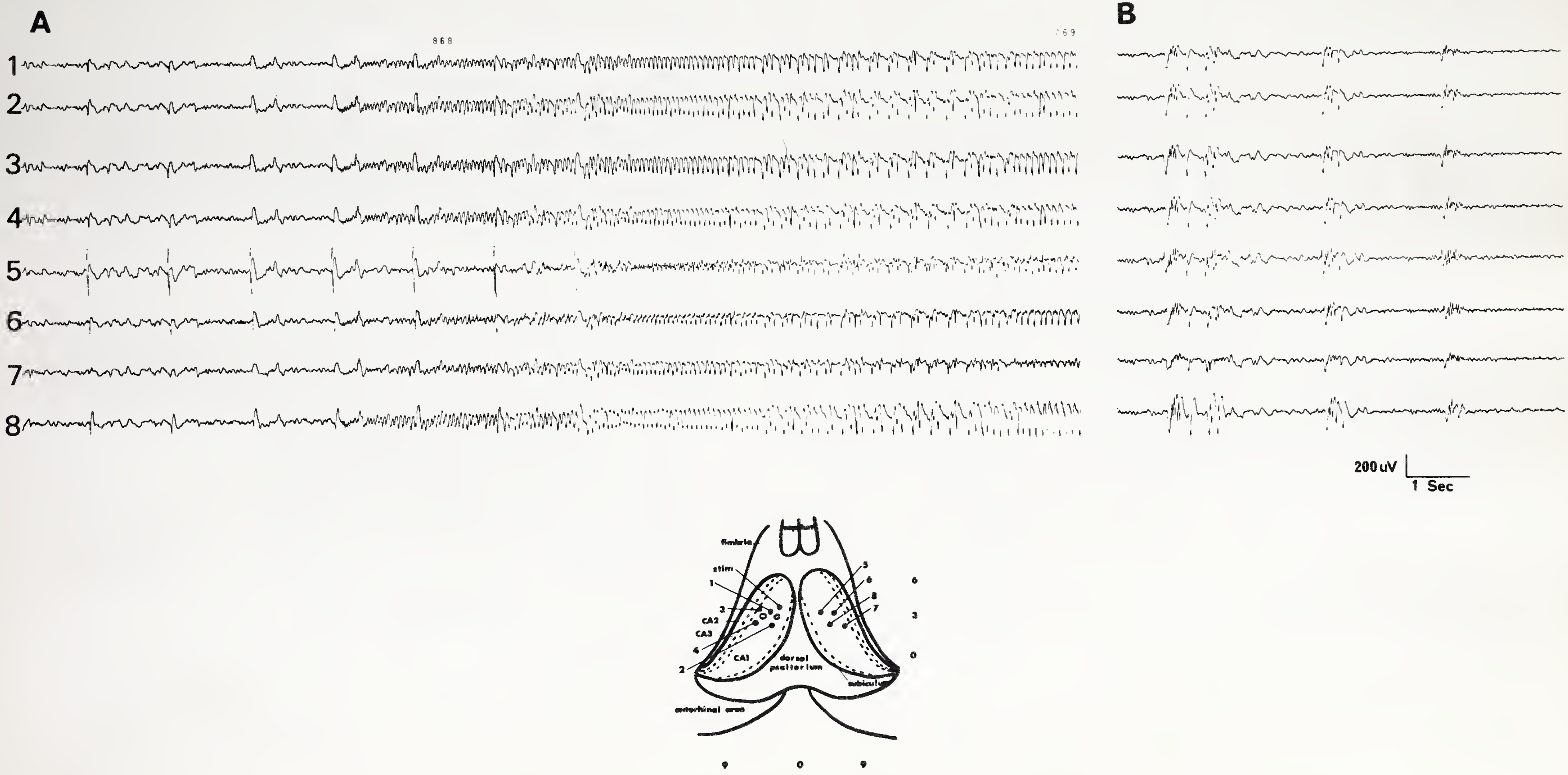
In seven experiments on four other cats, a sub-convulsive concentration of ouabain was perfused with a threshold concentration of high-potassium cerebrospinal fluid. Control experiments in which

Figure 9

Cat XII. Perfusion of a subthreshold concentration of potassium together with a subconvulsive concentration of ouabain.

A.) Onset of paroxysmal activity induced by single shocks 7 minutes after beginning the perfusion.

B.) Termination of the seizure showing characteristic bursts, gradually decreasing in amplitude.

A
868
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200uV
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dorsal psalterium
subiculum
entorhinal area
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9

4 - 5 perfusions of high-potassium cerebrospinal fluid, each leading to a seizure were performed consecutively at 20 minute intervals. A statistical analysis of the results was not made due to insufficient number, but it was our impression that latency of onset of the epileptic activity was markedly shortened when both potassium and ouabain were given together. The intensity of the seizure as judged by duration of abnormal electrical activity and violence of clinical manifestations also appeared strikingly enhanced.

In four additional experiments, the concentration of potassium necessary to induce paroxysmal activity was determined at 30 minutes and 60 minutes after perfusion with 10^{-6}M ouabain alone for 30 minutes. No changes in the potassium threshold, seizure pattern or intensity were observed.

D. Analysis of perfusion effluents

The results from flame photometric analysis of 41 perfusates are presented in Tables 4a-c. In all cases where ouabain was perfused alone in concentrations of 10^{-6} to 10^{-5}M, a rise in potassium was seen in the effluent when compared to the inflow. While this is an unequivocal finding, a mean value for the increase cannot be computed as conditions varied from experiment to experiment principally with regard to rate of flow and whether or not epileptic activity developed.

TABLE 4a

Results from flame photometric analysis of 14 high-potassium CSF perfusates

Inflow K	Outflow K	Δ K	Inflow Na	Outflow Na	Δ Na	Presence of paroxysmal activity
3.0	2.9	-0.1	122.5	124.0	1.5	No
3.1	2.9	-0.2	130.0	133.0	3.0	No
2.4	2.7	0.3	131.0	132.0	1.0	No
4.4	4.3	-0.1	129.7	130.0	0.3	No
11.1	11.3	0.2	109.0	121.0	12.0	No
11.5	11.1	-0.4	124.0	127.0	3.0	No
11.8	10.5	-1.3	109.0	110.0	1.0	No
13.7	12.4	-1.3	114.0	116.0	2.0	No
13.9	13.8	-0.1	109.0	110.0	1.0	Yes
16.7	16.9	0.2	117.0	120.0	3.0	Yes
16.8	16.8	0	112.0	118.0	6.0	Yes
17.3	17.5	0.2	113.0	116.0	3.0	Yes
18.3	14.8	-3.5	118.0	126.0	8.0	Yes
22.6	20.5	-2.1	120.0	122.0	2.0	Yes

TABLE 4b

Results from flame photometric analysis of 14 ouabain-CSF perfusates

Inflow K	Outflow K	Δ K	Inflow Na	Outflow Na	Δ Na	Conc. ouabain	Seizure activity
2.9	2.7	-0.1	125.0	127.0	2.0	10^{-7}	No
3.1	3.2	0.1	129.5	129.7	0.2	10^{-7}	No
2.7	3.1	0.4	127.1	128.0	0.9	5×10^{-7}	No
2.4	3.1	0.7	124.6	128.2	3.6	10^{-6}	No
2.2	2.7	0.5	127.0	127.0	0	10^{-6}	No
2.3	2.8	0.5	126.7	128.3	1.6	10^{-6}	No
2.5	3.0	0.5	125.0	126.7	1.7	10^{-6}	No
2.2	3.8	0.6	128.0	129.0	1.0	2×10^{-6}	No
2.9	3.7	0.8	125.3	127.0	1.7	7×10^{-6}	Yes
2.4	3.4	1.0	129.2	130.0	0.8	8×10^{-6}	Yes
2.9	3.7	0.8	127.0	128.0	1.0	10^{-5}	Yes
3.1	5.8	2.7	124.0	127.0	3.0	5×10^{-5}	Yes
2.8	3.5	0.7	126.0	126.5	0.5	5×10^{-5}	Yes
3.0	3.7	0.7	123.3	127.5	4.2	5×10^{-5}	Yes

TABLE 4c

Results from flame photometric analysis of high-K with ouabain-CSF in 13 perfusates

Inflow K	Outflow K	Δ K	Inflow Na	Outflow Na	Δ Na	Conc. ouabain	Seizure activity
9.5	9.6	0.1	121.0	124.0	3.0	10^{-6}	Yes
10.9	12.3	1.4	107.0	116.0	9.0	5×10^{-6}	Yes
11.2	9.9	-1.3	107.0	108.0	1.0	2×10^{-6}	Yes
11.6	11.3	-0.3	129.0	130.0	1.0	2×10^{-6}	Yes
12.8	13.0	0.2	116.0	127.0	11.0	10^{-6}	Yes
16.8	16.4	-0.4	111.0	118.0	7.0	5×10^{-7}	Yes
17.2	17.3	0.1	113.0	121.0	8.0	10^{-7}	Yes
17.6	17.4	-0.2	114.0	114.0	0	5×10^{-6}	Yes
18.0	17.0	-1.0	---	---	---	2×10^{-6}	Yes
19.2	18.0	-0.8	121.0	124.0	3.0	2×10^{-6}	Yes
19.2	18.0	-1.0	112.0	112.0	0	2×10^{-6}	Yes
23.6	21.2	-2.5	109.0	112.0	3.0	10^{-6}	Yes
24.0	23.7	-0.3	109.0	110.0	1.0	10^{-6}	Yes

When potassium alone or ouabain in combination with high potassium cerebrospinal fluid was perfused, there was either no change or more commonly, a decrease in effluent potassium concentration. Sodium levels were consistently higher in the outflow than in the inflow.

II. Cortical Preparations

A. General Behavior

This did not differ significantly from the description given above for the hippocampal preparations. Alterations in behavior following an experimental session were usually milder in these cats and of shorter duration.

B. Perfusion with ouabain and potassium

Thirteen experiments were performed on the eleven cats having cortical implantation of electrodes within a lucite chamber for perfusion. The rate of perfusion was maintained at 0.5ml/min. Table 5 gives a summary of the results.

In only seven experiments was high potassium cerebrospinal fluid effective in inducing epileptic activity. Significantly higher concentrations of potassium were required on cortex than in dorsal hippocampus. In only five experiments was ouabain alone able to induce seizure activity. Concentrations of 10^{-4}M were necessary in all instances. Experiments performed on the six cats in which abnormal discharges had been successfully elicited with high-potassium cerebrospinal fluid showed that ouabain in a subconvulsive concentration of 10^{-5}M inhibited the development of epileptic activity when perfused together with a concentration of potassium that had previously induced a seizure. In two cats, however, a second perfusion of threshold potassium was unable to provoke paroxysmal discharges. Raising

TABLE 5

K conc. (x normal)	No. Perfusions	Active perf. with SST*	Active perf. without SST	SST effective in inducing seizure	% active	Latency with SST	Latency without SST
K x 6	4	0	0	0	0	---	---
K x 12	7	1	1	0	28.6	7 - 8	7 - 8
K x 15	7	1	1	0	28.6	21 - 23	21 - 23
K x 18.5	7	1	---	0	14.3	14	---
K x 19.5	10	2	2	0	40.0	16 - 28	16 - 28
K x 22	11	2	1	0	27.3	16 - 42	16 - 42
K x 26	2	0	0	0	0	---	---

NB If a cat exhibited epileptic activity at one concentration of potassium, no higher concentrations were perfused _unless_ the second perfusion with the initial concentration failed to induce a seizure

* SST - single shock trains

the potassium concentration in these instances had no effect.

III. Post-mortem Examination of Perfused Brains

A. Hippocampal Preparations

By and large, pathological changes were limited to damage caused by insertion of the cannulas and electrodes and the continued presence in the brain of these foreign elements. Depending upon the length of time between the surgical implantation and the death of the animal various stages of tissue reaction were noticed. Invariably, marked capillary proliferation, microglial cell infiltration and increasing numbers of fibroblasts were seen along the electrode and cannula tracks. Myelin stains showed degenerating myelin immediately adjacent to the tracks. Fragmentation or almost complete disappearance of ependyma was observed in the perfused ventricles. Varying numbers of polymorphonuclear leukocytes and mononuclear leukocytes were observed in the tissue response. Occasionally microabscesses were observed in the cortical white matter and rarely there was evidence of a diffuse meningoencephalitis with ventriculitis.

The reaction in the hippocampus proprius was minimal. Except in the few cats which had received infusions of large amounts of ouabain (greater than 10ugms.) no changes in pyramidal or glial cell structure could be noted. There did not appear to be increased numbers of glial cells except in the tissue response around the electrodes. Where large infusions of ouabain

had been given, swelling of the neurons and glial cells was seen. The diffuse spongioform changes reported by Bignani and Palladini (7), Cornog et al (20), and Maccagnani et al. (64) were not seen.

B. Cortical Preparations

None of the brains removed from the cats with cortical electrodes were examined microscopically. In all cases, there was grossly evident an area of intense tissue reaction under the lucite chamber and sharply demarcated from the surrounding cortical surface. What appeared to be a dense inflammatory membrane covered the cortex under the chamber and could be peeled off with some difficulty. In a few cats there were cystic changes grossly evident beneath this membrane.

DISCUSSION

The inhibition of active transport by impressively small concentrations of cardiac glycosides was discovered by Schatzman in 1953 (78). Since that time, these drugs have served as potent weapons in the armamentarium of biologists investigating fundamental processes at a cellular level. They were instrumental in delineating the closeness between the sodium pump and Skou's enzyme system (29, 38,39,79,80,84,85). While the mechanism underlying their clinical usefulness in increasing the force of systolic contraction in the failing heart has as yet escaped analysis (67,101), the digitalis glycosides have contributed immensely to the unravelling of many of the mysteries involved in the basic functioning of excitable tissues. Ouabain in particular has been used widely to help define the ionic basis for the membrane potential and impulse propagation in isolated and peripheral nerve (6,15,46-48,49,57,92). Neuromuscular transmission and muscular contraction have been studied systematically using ouabain (21,31,34,50). This drug has also been employed in work on the superior cervical ganglion (9,59,72)

and the retina (32,60). As regards the central nervous system, numerous investigations have been performed using ouabain to elucidate the functioning of the Na-K ATPase in isolated slabs and microsomal fractions of cortex (26,33,35,36,44,61,65,73,76, 89). Most of these studies were directed specifically toward correlating ion concentrations, ATPase activity and brain metabolism.

In vivo work with ouabain has intensively explored factors involved in cerebrospinal fluid formation showing that an active pump mechanism is necessary for continued production of cerebrospinal fluid. Inhibition of this pump by ventriculocisternal perfusion of 10^{-5}M ouabain leads to near-complete cessation of cerebrospinal fluid secretion (12,22,24,55,69,95,98). Oppelt and Palmer have recently reported that lower doses of ouabain (10^{-9} to 10^{-14}M) have just the opposite effect and significantly stimulate cerebrospinal fluid formation with perfused ventriculocisternally. Other *in vivo* investigations have utilized ouabain to clarify the participation of active transport in the blood-brain barrier (12, 16,22,24,25,55,74). All of these experiments were performed on anesthetized acute preparations.

The effects of ouabain on cerebral electrical activity in living animals have been neglected by comparison. In 1966, Maccagnavi, Bignani and Palladini (64) and Bignani and Palladini (7) injected 0.3 - 0.6mg of ouabain into the cerebral cortex of rats and observed a stereotyped response almost immediately. EEG's

recorded from parietal and nasal bone leads showed seizures consisting of multiple spikes in rapid succession alternating with high voltage waves. Clinically the rats manifested a series of violent tonic-clonic convulsions which gradually evolved into status epilepticus. Their animals invariably died 2 - 3 hours after injection. Cornog, Gonatos and Feiermann (20) reported on the changes observed in cerebral electrical activity after injecting 5ugms of ouabain 3mm below the surface of a rat's fronto-parietal cortex. A typical response was consistently seen electrographically. Two minutes after injection rolling slow waves appeared. These were superceded at 11 minutes with a depressed background and occasional spikes which increased in frequency and amplitude over the next 25 minutes until a generalized seizure was generated. Just before the animal's death, high amplitude slow waves with superimposed small spikes appeared. Sedlacek (83) working on guinea pig embryos showed that 3.4×10^{-4}M ouabain had a depolarizing effect on cortical steady potentials. He compared these results to those obtained when 3.3M potassium was applied to the cortex and found only two slight differences: 1) Depolarization was observed at a younger gestational age with ouabain than with potassium and 2) quantitatively, the depolarization produced with ouabain was greater than with potassium. As the fetus approached term, the latent period before onset of depolarization decreased. Last year, Aquino-cias and his co-workers (4) implanted macroelectrodes in reticular formation, thalamus, caudate,

dorsal hippocampus and neocortex of cats. After the anesthesia had worn off, acute experiments on the succinylcholine-paralyzed animals were performed. After topical application of a piece of cotton soaked with 2.5mg ouabain to occipital cortex, they observed a significant decrease in the convulsive threshold to electro-shock stimulation and potentiation of the after-discharges seen following the seizure. In addition, spontaneous spikes appeared later and propagated to other cortical and subcortical areas becoming generalized. A 2% KCl solution applied in a similar manner appeared to give almost identical results but with a slightly different time course. When given together, results interpreted as additive were observed.

The results of the present investigation are in general agreement with the findings cited above. Localized perfusion of dorsal hippocampus with concentrations of ouabain 5×10^{-6}M or greater consistently produced characteristic epileptic activity. Caution in attaching significance to this absolute value as a "threshold" figure is warranted, however. The experimental method was such that during the course of a single experiment, successive perfusions with increasing concentrations were made until electrographic changes were recorded. As there is considerable evidence for long-lasting and cumulative effects due to the drug this value may actually represent an effective summation concentration. Numerous authors reporting on work in a variety of *in vitro* preparations encountered difficulty in attempting to reverse

ouabain's action once it is bound to tissue (6,15,32,35,60,89). Similarly many of these experiments show that the drug has a long duration of action — up to several hours in some instances (15,35). While these aspects were not systematically studied in the present investigation, there was a clear impression that ouabain was very slowly removed from perfused areas. Four observations support this. First, in three animals, abnormal EEGs were recorded up to 48 hours after the end of a perfusion which had induced paroxysmal activity. Changes from the normal consisted of periodic spikes and bursts of high amplitude waves occurring irregularly from the side which had been perfused. In all three of these cats, electrical activity subsequently returned to normal. Second, in four cats, after a given concentration of ouabain had produced a seizure and electrical activity had apparently returned to nromal for at least 30 minutes, a second perfusion was made using a lower concentration of ouabain which previously in the same cat had not led to the development of paroxysmal discharges. In all four animals, a new round of seizures was generated, more intense than before and leading, in two instances, to the cat's death. Third, ten cats were returned to their cages after having been given ouabain either by perfusion or infusion. All had apparently normal EEGs, yet were found dead 12 hours later. Only tow of these animals had received ouabain for the first time; the others had been used at least once before and in most cases for several experiments. This suggests a cumulative effect and/or diffusion to vital

mesencephalic structures. Finally, persistence of abnormal behavior for as long as two days after an experiment suggests either persistence of the drug itself in cerebral tissues or long-lasting consequences of an initial drug action.

There appeared to be no clearly defined relationship between the concentration of ouabain in the perfusion fluid, the latency of onset of seizure discharges, the intensity of the seizure, or even the survival of the animal. In the first series of experiments, therefore, where ouabain was being constantly perfused, it is conceivable that ouabain was being bound to membrane constituents and that more important than the concentration of ouabain was the exact amoung being removed by the tissues from the perfusion. And with the unavoidable variation in perfusion rates, it seems likely that the amount of ouabain active on neuronal structures fluctuated widely from one experiment to another even though identical concentrations were used.

In the second group of experiments where a known amount of ouabain was injected into the ventricle, both the cumulative effects of the drug and the uncertainty regarding the amount of ouabain actually effective may have been partly circumvented. There are several reasons for this. First, a fresh cat was used for the initial experiment at each amount tested. Second, only one infusion of an exactly known amount was made during each experimental session. And third, a ten-day interval was waited

between experiments on the same animal.* Thus, in this situation, three micrograms may indeed represent the minimal amount of ouabain which will induce paroxysmal activity in the hippocampus. It is noteworthy that the range between the amount necessary to induce minimal epileptic effects and that which led to prolonged violent seizures always terminating in death was very small: 3ugms vs. 7ugms.

To what extent the marked difference between concentrations of potassium required to induce epileptic activity in hippocampus and those necessary in neocortex reflect the well-known variance in the excitability of the two structures (51,62,75) cannot be surmised from this investigation. The pathological changes seen at autopsy on the cortical surface beneath the lucite chamber make any inferences hazardous. In such circumstances, conclusions based on results obtained with ouabain either alone on in combination with potassium are even more tenuous because of the unknown variable this drug represents. Thus, a confrontation with the apparent contradiction represented by the differences observed in the interaction of potassium and ouabain on neocortex as

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* When given systemically, urinary excretion of the unchanged glycoside appears to be the major means of removal from the body. Some conversion takes place in the liver with metabolites being found in the bile. One study showed that 20% of an intravenous dose of digitoxin could not be accounted for after seven days (101). Nothing is known of the means by which ouabain is cleared from cerebral tissues and the ten-day interval may well permit traces of the drug to remain in the brain.

opposed to hippocampus can be temporarily avoided, and a discussion of the experimental results derived from the cortical preparations postponed until further experiments can be performed yielding observations less equivocal.

The finding that ventricular perfusion of ouabain raises the concentrations of potassium in the effluent agrees with the results reported by Bradbury and Davson (12), Cserr (22), Cameron (16), and Katzman et al (55). Cserr had postulated that the increased levels of potassium were due to inhibition of an active transport process across the blood-brain barrier. Bradbury and Davson, on the other had, hypothesized that leakage of intracellular potassium into the cerebrospinal fluid from poisoned nerve cells accounted for the increase. Subsequent work by Cameron (17) suggests that both mechanisms may be involved. He lowered plasma potassium concentration below CSF levels by intravenous glucose and insulin, and rectal infusion of a cation exchange resin. When ouabain was then perfused ventriculocisternally, a rise in the effluent potassium still occurred but to a lesser degree, indicating the brain as the source of this portion of the increase. Next, serum potassium was elevated by KCl after a rise in CSF potassium had been induced by ouabain. A further increase was noted. Cameron interpreted this to mean that some part of the potassium elevation came from alterations in the blood-brain barrier.

The action of ouabain in inducing paroxysmal activity is most probably to its inhibitory action on the Na-K activated ATPase. The concentrations necessary to produce seizures support this conclusion as they correlate well with values obtained from _in vitro_ studies. Extracellular and intracellular concentrations of ions, primarily potassium and sodium, are altered in neuronal populations exposed to the drug. Most likely, both the increase in intracellular sodium and the increase in extracellular sodium and the increase in extracellular potassium are instrumental in driving membrane potentials of affected neurons toward zero, thus raising hippocampal excitability to epileptic levels. In a comprehensive study on the mechanism involved in ouabain's depolarizing action, Casteels (18) used intracellular microelectrodes to record membrane potentials from smooth muscle cells exposed to a bathing fluid containing 10^{-6}M ouabain. He also measured internal and external sodium and potassium concentrations. The fall in membrane potential with ouabain was greater than the equilibrium potential for potassium as predicted by the Nernst equation. His conclusion was that changes in a single ion's concentration would not explain the large shift in membrane potential and that both increased permeability to sodium influx and potassium efflux were involved. While difficult to deduce cellular events from macroelectrode recordings, it is tempting to infer that the differences in the electrographic patterns observed between ouabain-induced epileptic activity and potassium -

induced epileptic activity reflect this bimodal action. A more substantial piece of evidence that ouabain's action is not solely a consequence of elevation of extracellular potassium is our observation that single-shock trains elicited no convulsive response when delivered during perfusion with ouabain alone. Zuckermann and Glaser (102) have demonstrated that effects induced by single shocks are highly specific for a gradually increasing level of hippocampal excitability during perfusion with high - potassium cerebrospinal fluid. Whether our results indicate that onset of ouabain-induced paroxysmal activity is an "all-or-none" phenomenon occurring without a gradual rise in the exciatbility of perfused neurons, or whether the drug causes changes in neuronal somata cannot be decided on the basis of this study. It is significant that single-shock trains were nearly always successful in precipitating a seizure when delivered furing perfusion with potassium and ouabain together.

Another difficulty in attempting to make some correlation between the increased potassium seen after ouabain alone and onset of the induced epileptic activity is that the concentration measured in the outflow is the average concentration from a period of time during which constant dynamic changes are taking place between perfusion fluid, naturally formed CSF and intra- and extracellular compartments. While the rise in potassium after ouabain is most likely related to the drug, the increase in sodium seen not only with ouabain but with high-K CSF and ouabain-potassium

together, is not. Rather, this finding can probably best be explained as an equilibrium phenomenon occurring between relatively hyponatremic artificial CSF and extracellular fluids. This is almost certainly the case with perfusion where the sodium has been reduced secondary to an increased potassium concentration.

Unfortunately, too few experiments were performed in which both potassium and ouabain were perfused simultaneously to allow a definite statement to be made regarding their interaction on dorsal hippocampus. Nonetheless, the results strongly suggest that ouabain facilitates the action of potassium in inducing epileptic activity. This is demonstrated by onset of paroxysmal discharges at lower concentrations of potassium when combined with a subconvulsive concentration of ouabain than with potassium alone; a decrease in the time from beginning of perfusion to the appearance of spikes when threshold potassium is combined with subthreshold ouabain; and a seizure increased in length and intensity in this latter case. This is compatible both with the mechanism postulated to explain how ventricular perfusion with high-K CSF is able to induce epileptic activity (102) and the probable basis outlined above for ouabain's epileptogenic action on hippocampus. That is, facilitation of the potassium-induced changes is probably secondary to making the neurons more susceptible to excess potassium as a consequence of the direct action of ouabain in lowering the membrane potentials of the perfused neuronal elements. It is also possible that ouabain inhibits mechanisms normally

responsible for clearing excess potassium from extracellular fluids. Experiments studying Na-K ATPase have shown that high potassium levels can partially protect the enzyme against inhibition, but cannot reverse the effect once the drug is bound to membranes (5,85 for review). Clinically, potassium infusion has been used to combat life-threatening digitalis toxicity and the effectiveness of this procedure has been attributed to "antagonism" between the glycoside and potassium for enzyme sites (67). This is not necessarily antithetical to results obtained in this study. It is apparent that theoretically ouabain could be prevented from completely inhibiting ATPase by simultaneous perfusion with high concentrations of potassium, thus leading to a partial membrane depolarization rather than a complete depolarization block. The elevated potassium level in the perfusion fluid could then act upon this "sensitized" hippocampus to induce a seizure state.

The observations that ouabain reduces the concentration of potassium necessary to induce convulsive phenomenon and decreases the time before onset of seizure discharges, if confirmed, may provide a clue as to the reason for this latent period. Increases in extracellular potassium stimulate the active pumping mechanism. So long as greater work by this system can compensate for the potassium elevation and maintain membrane stability, epileptic activity is not seen. As the potassium concentration in the perfusion is raised (or the pump becomes less efficient as with ouabain) the perfused cells become less able to maintain their

normal electrical integrity and the end result is a seizure. Sufficient energy stores may be an additional governing factor.

Although there is little doubt that ouabain induces epileptic activity primarily through its inhibition of Na-K ATPase, there may be other factors contributing to and modifying the seizure phenomenon. Birks (9) has shown that there is an increased amount of acetylcholine released from cat sympathetic ganglia after application of ouabain. Through a series of carefully designed experiments he was able to conclude that accumulation of sodium in the presynaptic terminals as a consequence of ouabain was directly related to the augmented acetylcholine release. Elmqvist (31) made similar observations at the neuromuscular junction. The possibility that increased catecholamine release has been mentioned by Helfant and his team (43). Should Samson's hypothesis that ATPase is relatively more important in inhibitory than excitatory neurons prove correct, then differential poisoning by ouabain could result in an imbalance between neuronal elements with a hyperexcitable state predominant. Finally ouabain may cause actual structural changes in cellular constituents either as corollaries of its direct action or as sequellae to the increased sodium and water found intracellularly after exposure to ouabain (11,32). Any of these postulations could conceivably contribute to development of epileptic activity during ventricular perfusion with ouabain.

While it is admittedly unlikely that elevated ouabain levels sould be found in the cerebrospinal fluid of epileptic patients, this study of an experimental model has some bearing on the findings of Zuckermann and Glaser (102) in relation of epileptogenesis. Our results show that alteration of an active transport enzyme system in a small region of brain has great epileptogenic potential. Such a focal alteration in membrane Na-K ATPase, either as a result of some insult to the tissue or some genetically-determined aberration, could provide a basis for one kind of epileptic focus. This would be reflected in the acknowledged importance of cerebral ionic environment in seizure phenomena. Thus, small increases in potassium at this site and/or other stimuli ordinarily trivial could trigger paroxysmal activity.

CONCLUSIONS AND SUMMARY

Although ouabain has been used before to study the effects of an ATPase inhibitor on cerebral electrical activity, the present investigation differs significantly from previous work in several aspects. First, cats chronically implanted with perfusion cannula and electrodes were used, thus permitting a series of experiments to be performed on the same cat without interference by anesthesia or the acute trauma of the surgical implantation. Second, small concentrations of ouabain were used in lone with those found to inhibit effectively the Na-K activated ATPase and active transport in a variety of *in vitro* preparations. An attempt was made to correlate drug effect with the amount used. Third, intraventricular perfusion of a definite region of brain (dorsal hippocampus) approximated *in vitro* studies on isolated neuronal tissues. Fourth, close proximity of recording electrodes to the perfusion cannulas allowed focal changes in electrical activity to be observed. And finally, an attempt was made to clarify the mechanism of action of ouabain by combining the drug

with potassium, an ion whose effects on hippocampal excitability are well-known.

Findings from experiments on 34 hippocampal preparations can be summarized as follows:

1) Ouabain perfused in concentrations greater than 5 X 10^{-6}M consistently induced paroxysmal activity with focal electrical changes appearing first in the perfused hippocampus. Perfusion up to 45 minutes with concentrations of 10^{-6}M or smaller led to no electrographic changes.

2) When known amounts of ouabain were injected through a cannula into the inferior horn, epileptic activity was seen to develop with the latency of onset being inversely proportional to the amount used. The range between the minimal amount necessary to produce a seizure and that leading invariably to the death of the animal was small: 3.5ugms and 7ugms respectively.

3) Ouabain appears to facilitate the action of potassium in inducing paroxysmal events in the dorsal hippocampus. Sub-convulsive concentrations of ouabain lowered the threshold to potassium and often prolonged the seizure as well.

4) When ouabain was perfused alone intraventricularly, the concentration of potassium in the effluent was found to be consistently raised.

Eleven cats with cortical implantations were used for a similar set of experiments. Seizure threshold to ouabain as well

as potassium was markedly higher in cortex than in hippocampus. When the two drugs were perfused together, the results were equivocal and contradictory.

The action of ouabain in inducing paroxysmal activity is most probably related to its inhibitory action on the Na-K activated ATPase although other mechanisms may be involved as well. Extracellular and intracellular concentrations of ions, principally potassium and sodium are altered in neuronal populations exposed to the drug. By increasing intracellular sodium and decreasing intracellular potassium, the membrane potential is lowered and the neuronal elements rendered more excitable. Facilitation of potassium-induced epileptic activity can be hypothesized to be related to both inhibition of mechanisms normally responsible for clearing excess potassium from extracellular fluids and by making the neurons more susceptible to excess potassium as a consequence of the direct action of ouabain.

BIBLIOGRAPHY

1. Abdel-Latif, A.A., J. Brody, and H. Ramahi (1967): Studies on Na-K ATPase of the nerve endings and the appearance of electrical activity in developing rat brain; J. Neurochem. 14:1133-1141

2. Aldridge, W.N. (1962): ATPase in the microsomal fraction from rat brain; Biochem. J. 83:527-533

3. Andersen, Per (1960): Interhippocampal impulses III. Basal dendritic activation of CA3 neurons; Acta physiol. scan. 48:209-230

4. Aquino-cias, J., T. Harmony, E. Guma, A. Toro, R. Aneiros, N. Hernandez, G. Fernandez (1967): Effect of strophantine and ouabaine on cortical after-discharges; Arch. int. Pharmacodyn. 168:352-365

5. Auditore, J.V. (1964): Character of G-strophanthin inhibition of the Na-K ATPase; Arch. int. Pharmacodyn. 149:90-98

6. Baker, P.F., and J. Manil (1968): The rates of action of potassium and ouabain on the sodium pump in squid axons; Biochem. Biophys. Acta 150:328-330

7. Bignani, A., and Palladini, G. (1966): Experimentally produced cerebral status spongiosus and continuous pseudorhythmic EEG discharges with a membrane ATPase inhibitor in the rat; Nature 209:413-414

8. Bignani, A., G. Palladini and G. Venturini (1966): Effect of cardiazol on Na-K activated ATPase of rat brain *in vivo*; Brain Res. 1:413-414

9. Birks, I.R. (1963): The role of sodium ions in the metabolism of acetylcholine; Canad. J. Biochem. Physiol. 41:2573-2597

10. Blum, B., J. Magner, E. Bental, and E. Liban (1961): Electroencephalographic studies in cats with experimentally produced hippocampal epilepsy; Electroencephalog. Clin. Neurophysiol. 13:340-353

11. Bourke, R.S. and D.B. Tower (1966): Fluid compartmentation and electrolytes of cat cerebral cortex "*in vitro*" I; J. Neurochem. 13:1071-1097

12. Bradbury, M.W.B. and H. Davson (1965): The transport of potassium between blood, cerebrospinal fluid and brain; J. Physiol. 181:151-174

13. Brinley, F.J. (1963): Ion fluxes in the central nervous system; Int. Rev. Neurobiol. 5:183-242

14. Burgen, A.S.V., (1967): Effect of drugs on membrane transport; Proc. Roy. Soc. Med. 60:329-334

15. Caldwell, P.C. and R.D.Keynes (1959): The effect of ouabain on the efflux of sodium from a squid giant axion; J. Physiol. 148:8P-9P

16. Cameron, I.R. (1967): Respiratory response to injection of ouabain into cerebral ventricles; Res. Physiol. 3:55-63

17. Cameron, I.R. (1968): The effect of sodium salicylate and ouabain on the exchange of potassium between cerebrospinal fluid and blood; J. Physiol. 195:21P-22P

18. Casteels, R. (1966): The action of ouabain on the smooth muscle cells of the guinea pig's taenia coli; J. Physiol. 184:131-142

19. Colfer, H.F. and H.E. Essex (1947): Distribution of total electrolytes, sodium and potassium, in cerebral cortex in relation of experimental convulsions; Am. J. Physiol. 150:27-36

20. Cornog, J.L., N.K. Gonatas, and J.R. Feierman (1967): Effects of intracerebral injection of ouabain on the fine structure of rat cerebral cortex; Amer. J. Path. 51:573-590

21. Cross, S.B., R.D. Keynes, and R. Rybova (1965): The coupling of sodium efflux and potassium influs in frog muscle; J. Physiol. 181:865-880

22. Cserr, Helen (1965): Potassium exchange between cerebrospinal fluid, plasma and brain; Am.J. Physio. 209:1219-1226

23. Cummins, J.T. and H. McIlwain (1961): Electrical pulses and the potassium and other ions of isolated cerebral tissues; Biochem. J. 79:330-341

24. Davson, Hugh (1967): "Physiology of the Cerebrospinal Fluid" J. and A. Churchill, Ltd., London; 445 pages

25. Davson, H. and M. Pollay (1963): Influence of various drugs on the transport of ^{131}I and PAH across the cerebrospinal fluid-blood barrier; J. Physiol. 167:239-246

26. de Piras, M.M. and J.A. Zadunaisky (1965): Effect of potassium and ouabain on glucose metabolism by frog brain; J. Neurochem. 12:657-661

27. Domer, F.R. (1961): Transport of ^{42}K from blood to cerebrospinal fluid in cats; J. Physiol. 158:366-372

28. Duncan, C.J. (1965): Cation-permeability control and depolarization in excitabli cells; J. Theor. Biol. 8:403-418

29. Dunham, E.T. and I.M. Glynn (1961): ATPase activity and the active movement of alkali metal ions; J. Physiol. 156:274-293

30. Eidelberg, E.(1961): Hippocampal "dendritic" responses in rabbits; J. Neurophysiol. 24:521-533

31. Elmqvist, D. and D.S. Feldman (1965): Effects of sodium-pump inhibitory on spontaneous acetylcholine release at the neuromuscular junction; J. Physiol. 181:498-505

32. Frank, R N. and T.H. Goldsmith (1967): Effects of cardiac glycosides on electrical activity in isolated retina of frogs; J. Gen Physiol. 50:1585-1606

33. Fried, R. (1965): Na-K activated ATPase from spinal cord of normal and injured rats; J. Neurochem. 12:815-823

34. Gage, P.W. (1965): Effect of cardiac glycosides on neuromuscular transmission; Nature 205:84-85

35. Gardos, G. (1964): Effect of ouabain on the active potassium accumulation; Experientia 20:127-128

36. Gibbs, R., P.M. Roddy, and E. Titus (1965): Preparation, assay and properties of a Na-K requiring ATPase from beef brain; J. Biol. Chem. 240:2181-2187

37. Glaser, G.H. (1964): Sodium and seizures; Epilepsia 5:97-111

38. Glynn, I.M. (1957): The action of cardiac glycosides on sodium and potassium movements in human red blood cells; J. Physiol. 136:148-173.

39. Glynn, I.M. (1962): Activation of ATPase activity by external potassium and internal sodium; J. Physiol. 160:18P-19P

40. Green, J.D. (1964): The Hippocampus; Physiol. Rev. 44:561-608

41. Green, J.D. and A.A. Arduini (1954): Hippocampal electrical activity in arousal; J. Neurophysiol. 17:533-557

42. Harafuji, Takuro (1959): Experimental study on Ammon's Horn seizures; Folia Psych. Neurol. Jap. 13:33-62

43. Helfant, R.H., B.J. Scherlag and A.M. Damato (1968): Electrophysiological effects of direct current countershock before and after ouabain sensitization and after diphenylhydantoin desensitization in the dog; Circ. Res. 22:615-623

44. Hess, H.H. (1962): Effect of sodium and potassium on Mg-stimulated ATPase activity of brain; J. Neurochem. 9:613-621

45. Hodgkin, A.L. (1964): "The Conduction of the Nervous Impulse" Liverpool University Press, Liverpool; 108 pages

46. Hodgkin, A.L. and A.F. Huxley (1952): Movement of sodium and potassium during nervous activity; Cold Spring Harbor Symp. Quant. Biol. 17:43-52

47. Hodgkin, A.L. and A.F. Huxley (1952): Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo; J. Physiol. 116:449-472

48. Hodgkin, A.L. and A.F. Huxley (1952): A quantitative description of membrane current and its application to conduction and excitation in nerve; J. Physiol. 117:500-544

49. Hodgkin, A.L. and R.D. Keynes (1955): Active transport of cations in giant axons from Sepia and Loligo; J. Physiol. 128:28-60

50. Horowicz, P. and C.J. Gerber (1965): Effects of external potassium and strophanthidin on sodium fluxes in frog striated muscle; J. Gen. Physiol. 48:489-514

51. Kaada, B.R. (1951): Somato-motor, autonomic and electrocorticographic response to electrical stimulation of "rhinencephalic" and other structures in primates, cat and dog; Acta. Physiol. Scand. 24 (Suppl. 83):1-263

52. Kandel, E.R., W.A. Spencer, and F.J. Brinley (1961): Transient and long-lasting electrical responses to direct hippocampal stimulation; Am. J. Physiol. 198:686-692

53. Kandel, E.R., W.A. Spencer, and F.J. Brinley (1961): Electrophysiology of hippocampal neurons: sequential invasion and synaptic organization; J. Neurophysiol. 24:225-242

54. Katz, B. (1966): "Nerve, muscle and synapse" McGraw-Hill Book Co. New York; 191 pages

55. Katzman, R., L. Graziani, R. Kaplan and A. Escriva (1965): Exchange of cerebrospinal fluid potassium with blood and brain: study in normal and ouabain perfused cats; Arch. Neurol. 13:513-524

56. Keesey, J.C. and H. Wallgren (1965): Movements of radioactive sodium in cerebral cortex slices in response to electrical stimulation; Biochem. J. 95:301-310

57. Keynes, R.D. and J.M. Ritchie (1965): The movements of labelled ions in mammalian non-myelinated nerve fibers; J. Physiol. 179:333-367

58. Koch, A. and D.M. Woodbury (1960): Carbonic anhydrase inhibition and brain electrolyte composition; Am.J. Physiol. 198:434-440

59. Konzett, H. and E. Rothlin (1952): Effect of cardioactive glycosides on a sympathetic ganglion; Arch. Int. Pharmacodyn. 89:343-352

60. Langham, M.E., S. Ryan, and M. Kostelnik (1967): Na, K-ion dependent ATPase of retina and mechanism of visual loss caused by cardiac glycosides; Life Sci. 6:2037-2047

61. Lewin, E. and A. Mc Crimmon (1967): ATPase activity in discharging cortical lesions induced by freezing; Arch. Neurol. 16:321-325

62. Liberson, W.T. and K. Akert (1953): Observations on electrical activity of the hippocampus, thalamus, striatum and cortex under resting conditions and during experimental seizure states in the guinea pig; Electroencephalog. Clin. Neurophysiol. 5:320

63. Liberson, W.T. and K. Akert (1955): Hippocampal seizure states in guinea pig; Electroencephalog. Clin. Neurophysiol. 7:211-222

64. Maccagnani, F., A. Bignani, and G. Palladini (1966): Etude electroencephalographique des effet de l'introduction intracranienne de ouabaine chez le rat et le cobaye; mise en evidence d'un trace periodique; Revue Neurologique 115:211-222

65. McIlwain, Henry (1963): "Chemical Exploration of the Brain" Elsevier Publishing Co. New York; 207 pages

66. Meyer, J.S., F. Gotoh, and Y. Tazaki (1961): Inhibitory action of CO_2 and acetazolamide in seizure activity; Electroenceph. Clin. Neurophysiol. 13:762-775

67. Moe, G.K. and A.E. Farah (1965): Digitalis and Allied Cardiac Glycosides; in "The Pharmacological Basis of Therapeutics" L.S. Goodman and A. Gilman, eds. 3rd edition; Macmillian Co. New York; 665-698

68. Nicholls, D., J. Kanfer and E. Titus (1962): Effect of ouabain on an incorporation of inorganic P^{32} into phospholipid; J. Biol. Chem. 237:1043-1049

69. Oppelt, W.W. and R.F. Palmer (1966): Stimulation of cerebrospinal fluid production by low doses of intraventricular ouabain; J. Pharm. Exp. Ther. 154:581-585

70. Palmer, R.F., K.C. Lasseter, and S.L. Melvin (1966): Stimulation of sodium and potassium dependent ATPase by ouabain; Arch. Biochem. Biophys. 113:629-633

71. Pappius, H. and K.A.C. Elliot (1954): Adenosine triphosphatase, electrolytes and O_2 uptake rates of human normal and epileptogenic cerebral cortex; Canad. J. Biochem. Physiol. 32:484-490

72. Perry, W.L.M. and H. Reinert (1954): The action of cardiac glycosides on autonomic ganglia; Brit. J. Pharmacol. 9: 324-328

73. Rawson, M.D. and J.H. Pincus (1968): The effect of diphenylhydantoin on sodium, potassium, magnesium-activated adenosinetriphosphatase in microsomal fraction of rat and guinea pig brain and on whole homogenates of human brain; Biochem. Pharmacol. 17:573-579

74. Reed, D.J., D.M. Woodbury, L. Jacobs and R. Squires (1965): Factors affecting distribution of iodide in brain cerebrospinal fluid; Am. J. Physiol. 209:757-764

75. Renshaw, B., A. Forbes, and B.R. Morrison (1940): Activity of isocortex and hippocampus. Electrical studies with microelectrodes; J. Neurophysiol. 3:74-105

76. Samaha, F.J. (1967): Studies of Na-K stimulated ATPase of human brain; J. Neurochem. 14:333-341

77. Samson, F.E. (1965): The Na-K activated ATPase as a mechanism for inhibitor neurons; Life Sci. 4:2243-2247

78. Schatzmann, H.J. (1953): Herzglykoside als Hemmstoffe für den aktiven K und Na transport durch die erythrocytemembran; Helv. Physiol. Acta 11:346-354

79. Schatzmann, H.J. (1965): The role of sodium and potassium in the ouabain inhibition of the Na-K activated membrane ATPase; Biochem. Biophys. Acta. 94:89-96

80. Schatzmann, H.J. and B. Räss (1965): Hemmung des aktiven Na-K transports und der Na-K aktivierten membran ATPase von erythrocytenstromata durch ouabain; Helv. Physiol. Pharmacol. Acta. 23:C47-C49

81. Schmidt, R.P., L.B. Thomas and A.A. Ward (1959): The hyperexcitable neuron. Microelectrode studies of chronic epileptic foci in monkeys; J. Neurophysiol. 22:285-296

82. Schwartz, A., H.S. Bachelard, and H. McIlwain (1962): The sodium-activated ATPase activity and other properties of cerebral microsomal fractions and sub-fractions; Biochem. J 84:626-637

83. Sedlacek, J. (1967): The effect of potassium and strophanthin-G upon cortical steady potentials in guinea pig embryos; Physiol. Bohe. 16:342-349

84. Skou, J.C. (1957): The influence of some cations on an ATPase from peripheral nerves; Biochem. Biophys. Acta. 23:394-401

85. Skou, J.C. (1965): Enzymatic basis for active transport of sodium and potassium across cell membrane; Physiol. Rev. 45:596-617

86. Skou, J.C. and C. Hilberg (1965): The effect of sulphydrul blocking reagents and urea on the (Na-K)-activated enzyme system; Biochim. Biophys. Acta 110:359-369

87. Snider, R.S. and W.T. Niemer (1961): "A Stereotaxic Atlas of the Cat Brain" University of Chicago Press, Chicago

88. Solomon, A.K., T.J. Gill, and G.L. Gold (1956): The kinetics of cardiac glycoside inhibition of potassium transport in human erythrocytes; J. Gen. Physiol. 40:327-350

89. Swanson, P.D. and H. McIlwain (1965): Inhibition of sodium - stimulated ATPase after treatment of isolated guinea pig cerebral cortex with ouabain and other agents; J. Neurochem. 12:877-892

90. Toman, J.E.P. (1965): Drugs Effective in Convulsive Disorders; in "The Pharmacological Basis of Therapeutics"; L.S. Goodman and A. Gilman, eds. 3rd edition; Macmillan Co. New York; 215-236

91. Tower, D.B. (1960): "Neurochemistry of Epilepsy" C.C. Thomas, Springfield; 335 pages

92. Treherne, J.E. (1966): Efflux of sodium ions in nerve cords of insects; J. Exptl. Biol. 44:355-362

93. Van Harreveld, A. (1966): "Brain Tissue Electrolytes" Butterworths, Washington; 171 pages

94. Van Harreveld, A. and J.P. Schade (1962): Changes in the electrical conductivity of cerebral cortex during seizure activity; Exptl. Neurol. 5:383-400

95. Vates, T.S., S.L. Bonting, and W.W. Oppelt (1964): Na-K activated ATPase formation of cerebrospinal fluid in the cat; Am.J. Physiol. 206:1165-1172

96. Ward, Arthur A.(1961): Epilepsy; Int. Rev. Neurobiol. 3:137-186

97. Ward, Arthur A.(1961): The eipleptic neuron; Epilepsia 2:70-80

98. Welch, K. (1963): Secretion of cerebrospinal fluid by choroid plexus of the rabbit; Am. J. Physiol. 205:617-624

99. Woodbury, D.M. (1955): Effect of diphenylhydantoin on electrolytes and radioactive sodium turnover in brain and other tissues of normal, hyponatremic and post-ictal rats; J. Pharmacol. Exp. Ther. 115:74-95

100. Woodbury, D.M., L.T. Rollins, M.D. Gardner, W.L. Hirschi, J.R. Hogan, M.L. Rallison, G.S. Tanner and S.A. Brodie (1958): Effects of CO_2 on brain excitability and electrolytes; Am. J. Physiol. 192:79-90

101. Wright, S.E. (1960): "The Metabolism of Cardiac Glycosides" Charles C. Thomas, Springfield; 86 pages

102. Zuckermann, E.C. and G.H. Glaser (1960): Hippocampal epileptic activity induced by localized ventricular perfusion with high-potassium cerebrospinal fluid; Exptl. Neurol. 20:87-110

103. Zuckermann, E.C. and G.H. Glaser (1968): Changes in hippocampal - evoked responses induced by localized perfusion with high - potassium cerebrospinal fluid; Exptl. Neurol. 22:96-117

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